HETEROCYCLIC COMPOUNDS AS CCR5 ANTAGONISTS

BACKGROUND OF THE INVENTION

The human immunodeficiency virus ("HIV") is the causative agent for acquired immunodeficiency syndrome ("AIDS"), a disease characterized by the destruction of the immune system, particularly of $CD4^+$ T-cells, with attendant susceptibility to opportunistic infections, and its precursor AIDS-related complex ("ARC"), a syndrome characterized by symptoms such as persistent generalized lymphadenopathy, fever and weight loss.

In addition to CD4, HIV requires a co-receptor for entry into target cells. The chemokine receptors function together with CD4 as co-receptors for HIV. The chemokine receptors CXCR4 and CCR5 have been identified as the main co-receptors for HIV-1. CCR5 acts as a major co-receptor for fusion and entry of macrophage-tropic HIV into host cells. These chemokine receptors are thought to play an essential role in the establishment and dissemination of an HIV infection. Therefore, CCR5 antagonists are thought to be useful as therapeutic agents active against HIV.

We have now discovered a series of small molecule nonpeptide compounds that are useful as inhibitors of HIV replication.

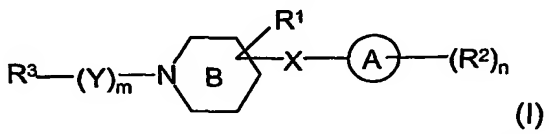
BRIEF DESCRIPTION OF THE INVENTION

The present invention features compounds that are useful in the inhibition of HIV replication, the prevention of infection by HIV, the treatment of infection by HIV and in the treatment of AIDS and/or ARC, either as pharmaceutically acceptable salts or pharmaceutical composition ingredients. The present invention further features methods of treating AIDS, methods of preventing infection by HIV, and methods of treating infection by HIV as monotherapy or in combination with other antivirals, anti-infectives, immunomodulators, antibiotics or vaccines. The present invention also features pharmaceutical compositions, comprising the above-mentioned compounds that are suitable for the prevention or treatment of CCR5-related diseases and

conditions. The present invention further features processes for making the above-mentioned compounds.

SUMMARY OF THE INVENTION

The present invention includes compounds of formula (I)



(I)

and pharmaceutically acceptable derivatives thereof, wherein:

X is a C_{1-5} alkylene chain, wherein said X is optionally substituted by one or more =O, =S, $-S(O)_t-$, alkyl, or halogen and wherein said C_{1-5} alkylene chain may optionally have 0-3 heteroatoms selected from oxygen, phosphorus, sulfur, or nitrogen;

Ring A is a saturated, partially saturated or aromatic 3-7 monocyclic or 8-10 membered bicyclic ring having one ring nitrogen and 0-4 additional heteroatoms selected from oxygen, phosphorus, sulfur, or nitrogen;

Ring B has an oxygen atom in addition to the depicted nitrogen;

R^1 is alkyl optionally substituted by one or more R^7, alkenyl optionally substituted by one or more R^7, alkynyl optionally substituted by one or more R^7, cycloalkyl optionally substituted by one or more R^8, heterocyclyl optionally substituted by one or more R^8, heteroaryl optionally substituted by one or more R^6, or aryl optionally substituted by one or more R^6; or R^1 and X taken together form a saturated, partially saturated or aromatic 5-6 memebered ring having 0-3 heteroatoms selected from oxygen, phosphorus, sulfur, or nitrogen that is fused to Ring A;

each R^2 is independently selected from $-OR^0$, $-C(O)-R^0$, $-S(O)_2-R^0$, $-C(O)-N(R^0)_2$, $-S(O)_2-N(R^0)_2$, $-(CH_2)_a-N(R^0)(-V_b-R^+)$, $-(CH_2)_a-(-V_b-R^+)$, halogen, alkyl optionally substituted by one or more R^7, alkenyl optionally substituted by one or more R^7, alkynyl optionally substituted by one or more R^7, aryl optionally substituted by one or more R^6, heteroaryl optionally substituted by one or more R^6, cycloalkyl optionally substituted by one or more R^8, or heterocyclyl optionally substituted by one or more R^8; and two adjacent R^2s on Ring A are optionally taken together to form a fused, saturated, partially saturated or aromatic 5-6 membered ring having 0-3 heteroatoms selected from oxygen, phosphorus, sulfur, or nitrogen; or two geminal R^2s are optionally taken together to form a spiro, saturated, partially saturated or aromatic 5-6 membered ring having 0-3 heteroatoms selected from oxygen,

phosphorus, sulfur, or nitrogen, said fused or spiro ring being optionally substituted by one or more R^8;

each a independently is 0-3;

each b independently is 0 or 1;

V is -C(O)-, -C(O)O-, $-S(O)_2-$, or $-C(O)-N(R^0)-$;

R^+ is alkyl, cycloalkyl, aralkyl, aryl, heteroaryl, heteroaralkyl, or heterocyclyl, wherein said R^+ is optionally substituted by one or more R^8;

m is 0 or 1;

n is 0-5;

R^3 is H, $-N(R^0)_2$, $-N(R^0)C(O)R^0$, -CN, halogen, CF_3, alkyl optionally substituted by one or more groups selected from R^7 or -S-aryl optionally substituted by $-(CH_2)_{1-6}-N(R^0)SO_2(R^0)$, alkenyl optionally substituted by one or more groups selected from R^7 or -S-aryl optionally substituted by $-(CH_2)_{1-6}-N(R^0)SO_2(R^0)$, alkynyl optionally substituted by one or more groups selected from R^7 or -S-aryl optionally substituted by $-(CH_2)_{1-6}-N(R^0)SO_2(R^0)$, cycloalkyl or carbocyclyl optionally substituted by one or more R^8, aryl optionally substituted by one or more R^6, heteroaryl optionally substituted by one or more R^6, or heterocyclyl optionally substituted by one or more R^8;

Y is alkyl, alkenyl, alkynyl, $-(CR^4R^5)_p-$, -C(O)-, -C(O)C(O)-, -C(S)-, $-O-(CH_2)_{0-4}-C(O)-$, $-(CH_2)_{0-4}-C(O)-O-$, $-N(R^0)-C(O)-$, $-C(O)-N(R^0)-$, $-N(R^0)-C(S)-$, $-S(O)_t-$, -O-C(=N-CN)-, $-O-C(=N-R^0)-$, $-C(=N-CN)-O-$, $-C(=N-R^0)-O-$, $-C(=N-CN)-S-$, -S-C(=N-CN)-, $-N(R^0)-C(=N-CN)-$, $-C(=N-CN)-$, $-N(R^0)-C[=N-C(O)-R^0]$, $-N(R^0)-C[=N-S(O)_t-R^0]$, $-N(R^0)-C(=N-OR^0)-$, $-N(R^0)-C(=N-R^0)-$, or $-C(=N-R^0)-$;

each R^4 is independently H, alkyl optionally substituted by R^7, alkenyl optionally substituted by R^7, or alkynyl optionally substituted by R^7;

each R^5 is independently selected from H, $-C(O)-OR^6$, $-C(O)-N(R^0)_2$, $-S(O)_2-N(R^0)_2$, $-S(O)_2-R^0$, aryl optionally substituted by R^6, or heteroaryl optionally substituted by R^6;

p is 1-5;

each t independently is 1 or 2;

each R^6 is independently selected from halogen, $-CF_3$, $-OCF_3$, $-OR^0$, $-(CH_2)_{1-6}-OR^0$, $-SR^0$, $-(CH_2)_{1-6}-SR^0$, $-SCF_3$, $-R^0$, methylenedioxy, ethylenedioxy, $-NO_2$, -CN, $-(CH_2)_{1-6}-CN$, $-N(R^0)_2$, $-(CH_2)_{1-6}-N(R^0)_2$, $-NR^0C(O)R^0$, $-NR^0(CN)$, $-NR^0C(O)N(R^0)_2$, $-NR^0C(S)N(R^0)_2$, $-NR^0CO_2R^0$, $-NR^0NR^0C(O)R^0$, $-NR^0NR^0C(O)N(R^0)_2$, $-NR^0NR^0CO_2R^0$, $-C(O)C(O)R^0$, $-C(O)CH_2C(O)R^0$, $-(CH_2)_{0-6}CO_2R^0$, $-O-C(O)R^0$, $-C(O)R^0$,

$-C(O)N(R^0)N(R^0)_2$, $-C(O)N(R^0)_2$, $-C(O)N(R^0)OH$, $-C(O)N(R^0)SO_2R^0$, $-OC(O)N(R^0)_2$, $-S(O)_tR^0$, $-S(O)_t-OR^0$, $-S(O)_tN(R^0)C(O)R^0$, $-S(O)_tN(R^0)OR^0$, $-NR^0SO_2N(R^0)_2$, $-NR^0SO_2R^0$, $-C(=S)N(R^0)_2$, $-C(=NH)-N(R^0)_2$, $-(CH_2)_{1-6}-C(O)R^0$, $-C(=N-OR^0)-N(R^0)_2$, $-O-(CH_2)_{0-6}-SO_2N(R^0)_2$, $-(CH_2)_{1-6}NHC(O)R^0$, or $-SO_2N(R^0)_2$ wherein the two R^0s on the same nitrogen are optionally taken together to form a 5-8 membered saturated, partially saturated, or aromatic ring having additional 0-4 heteroatoms selected from oxygen, phosphorus, nitrogen, or sulfur;

each R^7 is independently selected from halogen, $-CF_3$, $-R^0$, $-OR^0$, $-OCF_3$, $-(CH_2)_{1-6}-OR^0$, $-SR^0$, $-SCF_3$, $-(CH_2)_{1-6}-SR^0$, aryl optionally substituted by R^6, methylenedioxy, ethylenedioxy, $-NO_2$, $-CN$, $-(CH_2)_{1-6}-CN$, $-N(R^0)_2$, $-(CH_2)_{1-6}-N(R^0)_2$, $-NR^0C(O)R^0$, $-NR^0$ (CN), $-NR^0C(O)N(R^0)_2$, $-N(R^0)C(S)N(R^0)_2$, $-NR^0CO_2R^0$, $-NR^0NR^0C(O)R^0$, $-NR^0NR^0C(O)N(R^0)_2$, $-NR^0NR^0CO_2R^0$, $-C(O)C(O)R^0$, $-C(O)CH_2C(O)R^0$, $-(CH_2)_{0-6}-CO_2R^0$, $-C(O)R^0$, $-C(O)N(R^0)N(R^0)_2$, $-C(O)N(R^0)_2$, $-C(O)N(R^0)OH$, $-OC(O)R^0$, $-C(O)N(R^0)SO_2R^0$, $-OC(O)N(R^0)_2$, $-S(O)_tR^0$, $-S(O)_t-OR^0$, $-S(O)_tN(R^0)C(O)R^0$, $-S(O)_tN(R^0)OR^0$, $-NR^0SO_2N(R^0)_2$, $-NR^0SO_2R^0$, $-C(=S)N(R^0)_2$, $-C(=NH)-N(R^0)_2$, $-(CH_2)_{1-6}-C(O)R^0$, $-C(=N-OR^0)-N(R^0)_2$, $-O-(CH_2)_{0-6}-SO_2N(R^0)_2$, $-(CH_2)_{1-6}-NHC(O)R^0$, or $-SO_2N(R^0)_2$ wherein the two R^0s on the same nitrogen are optionally taken together to form a 5-8 membered saturated, partially saturated, or aromatic ring having additional 0-4 heteroatoms selected from oxygen, phosphorus, nitrogen, or sulfur;

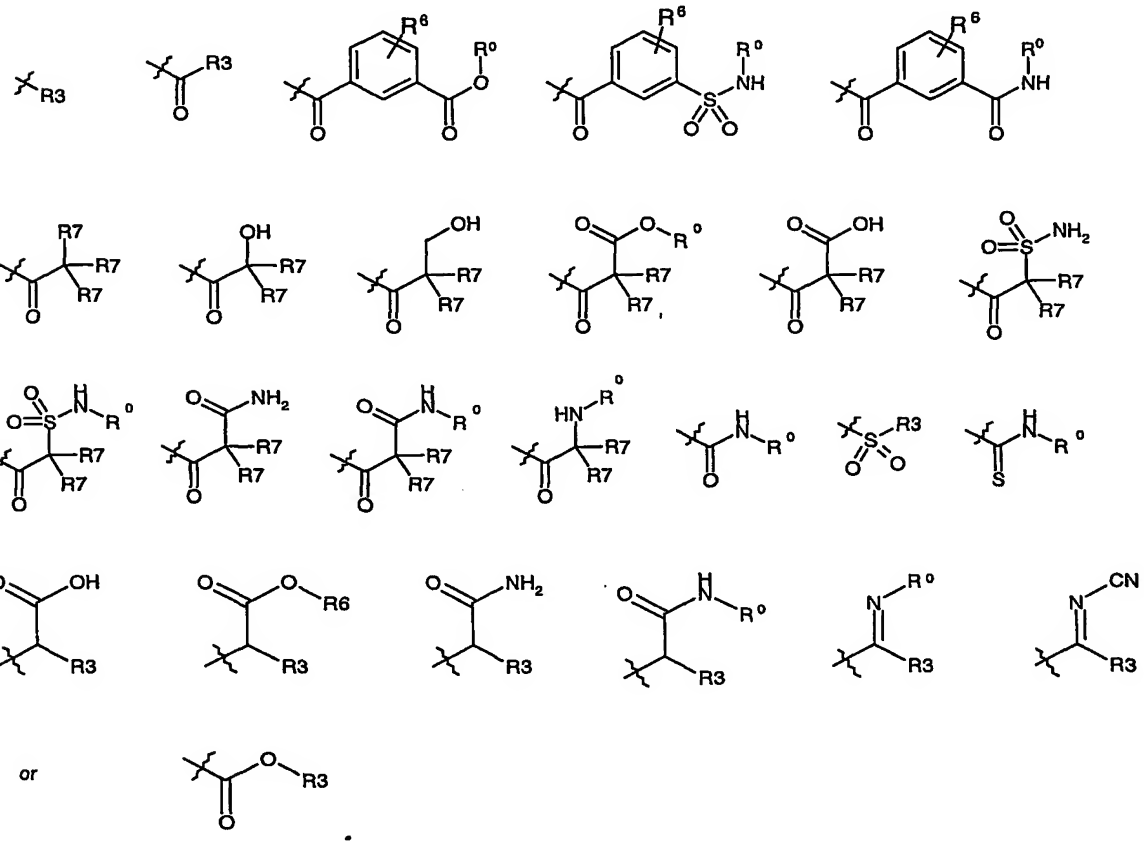
each R^8 is independently selected from R^7, =O, =S, $=N(R^0)$, =N(CN);

each R^0 is independently selected from hydrogen, alkyl, alkenyl, alkynyl, cycloalkyl, carbocyclylalkyl, aryl, heteroaryl, aralkyl, heteroaralkyl, heterocyclyl, or heterocyclylalkyl, wherein each member of R^0 except H is optionally substituted by one or more R*, OR*, $N(R^*)_2$, =O, =S, halo, CF_3, NO_2, CN, $-C(O)R^*$, $-CO_2R^*$, -C(O)-aryl, -C(O)-heteroaryl, -C(O)-aralkyl, $-S(O)_t$-aryl, $-S(O)_t$-heteroaryl, $-NR^*SO_2R^*$, $-NR^*C(O)R^*$, $-NR^*C(O)N(R^*)_2$, $-N(R^*)C(S)N(R^*)_2$, $-NR^*CO_2R^*$, $-NR^*NR^*C(O)R^*$, $-NR^*NR^*C(O)N(R^*)_2$, $-NR^*NR^*CO_2R^*$, $-C(O)C(O)R^*$, $-C(O)CH_2C(O)R^*$, $-C(O)N(R^*)N(R^*)_2$, $-C(O)N(R^*)_2$, $-C(O)NR^*SO_2R^*$, $-OC(O)N(R^*)_2$, $-S(O)_tR^*$, $-NR^*SO_2N(R^*)_2$, $-SO_2N(R^*)_2$ wherein the two R*s on the same nitrogen are optionally taken together to form a 5-8 membered saturated, partially saturated or aromatic ring having additional 0-4 heteroatoms selected from oxygen, phosphorus, nitrogen or sulfur; and

each R* is independently H, alkyl, alkenyl, alkynyl, cycloalkyl, aryl, or heteroaryl.

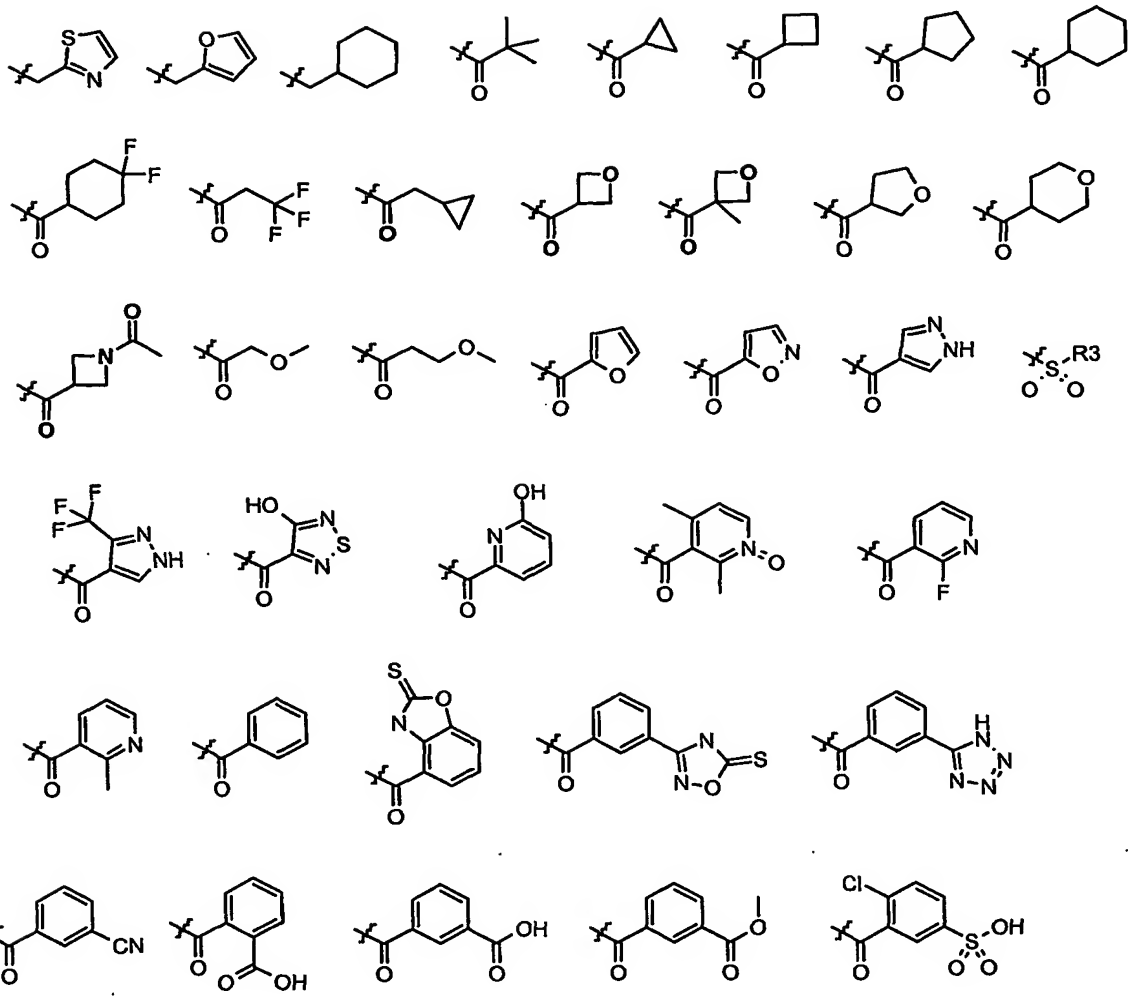
In one embodiment R^1 is optionally substitued aryl, such as R^1 is phenyl mono- or di- substituted with halogen, such as R^1 is phenyl di-substituted with Cl.

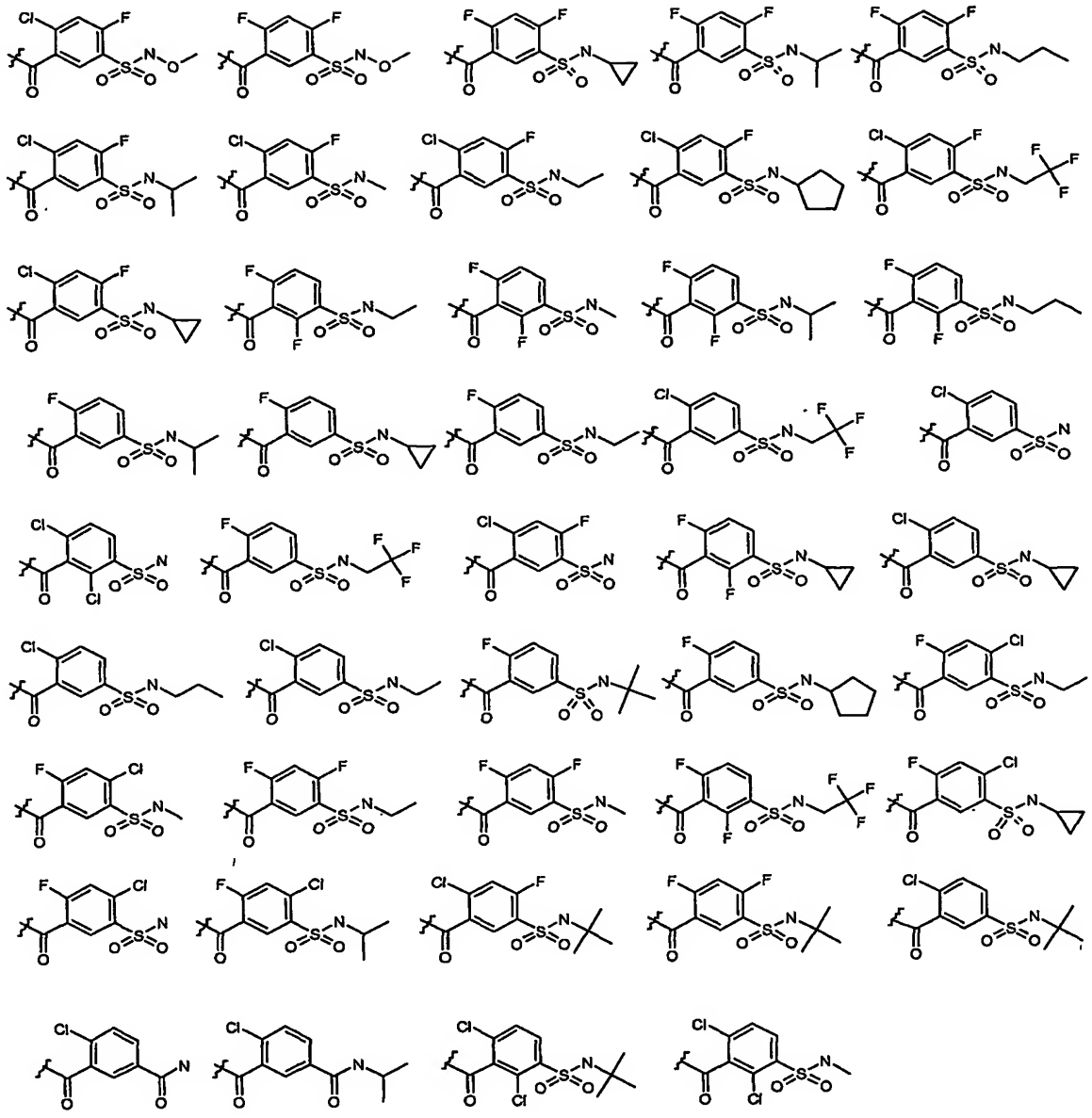
In one embodiment $-(Y)_m-R^3$ suitably is

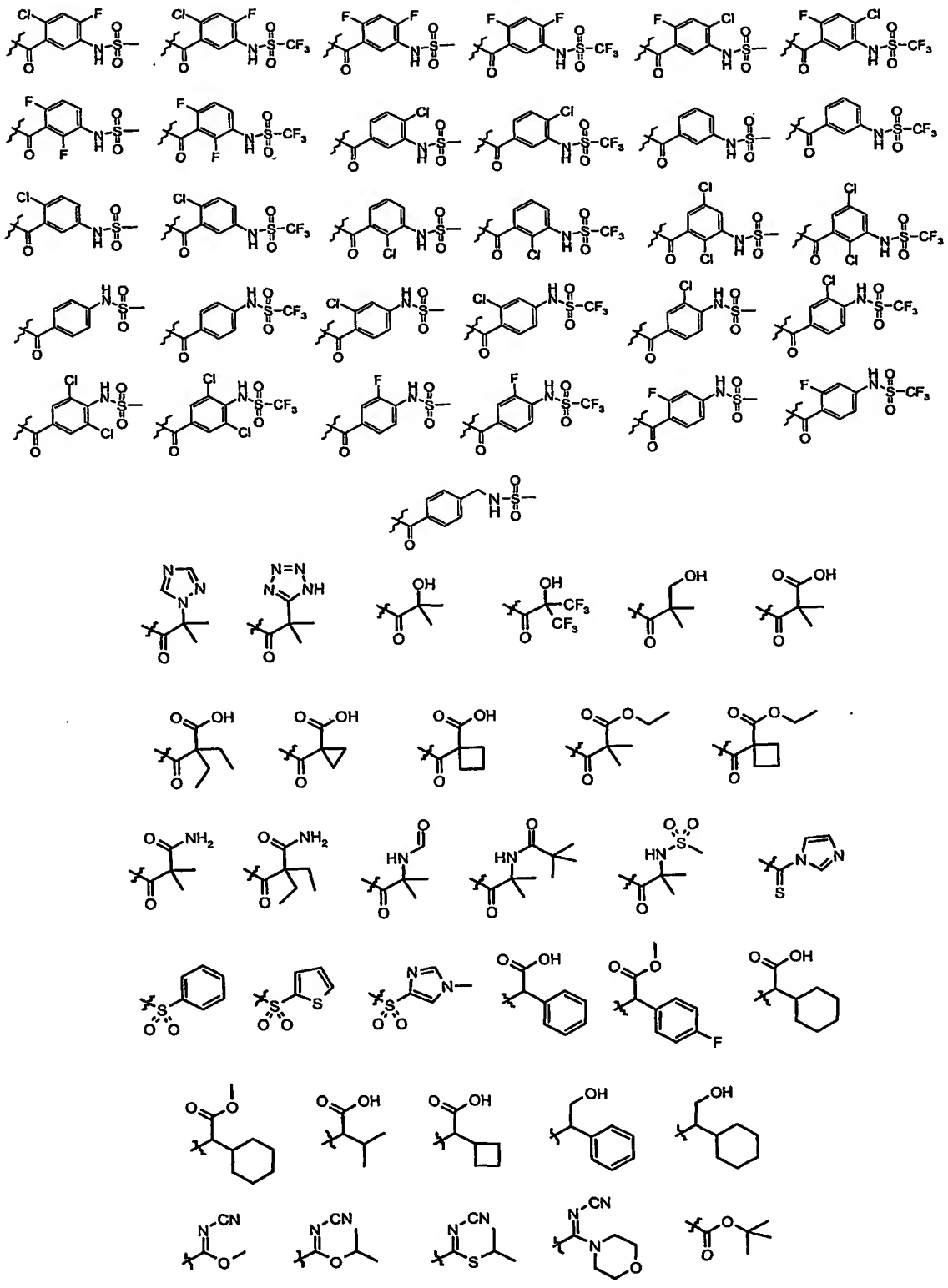


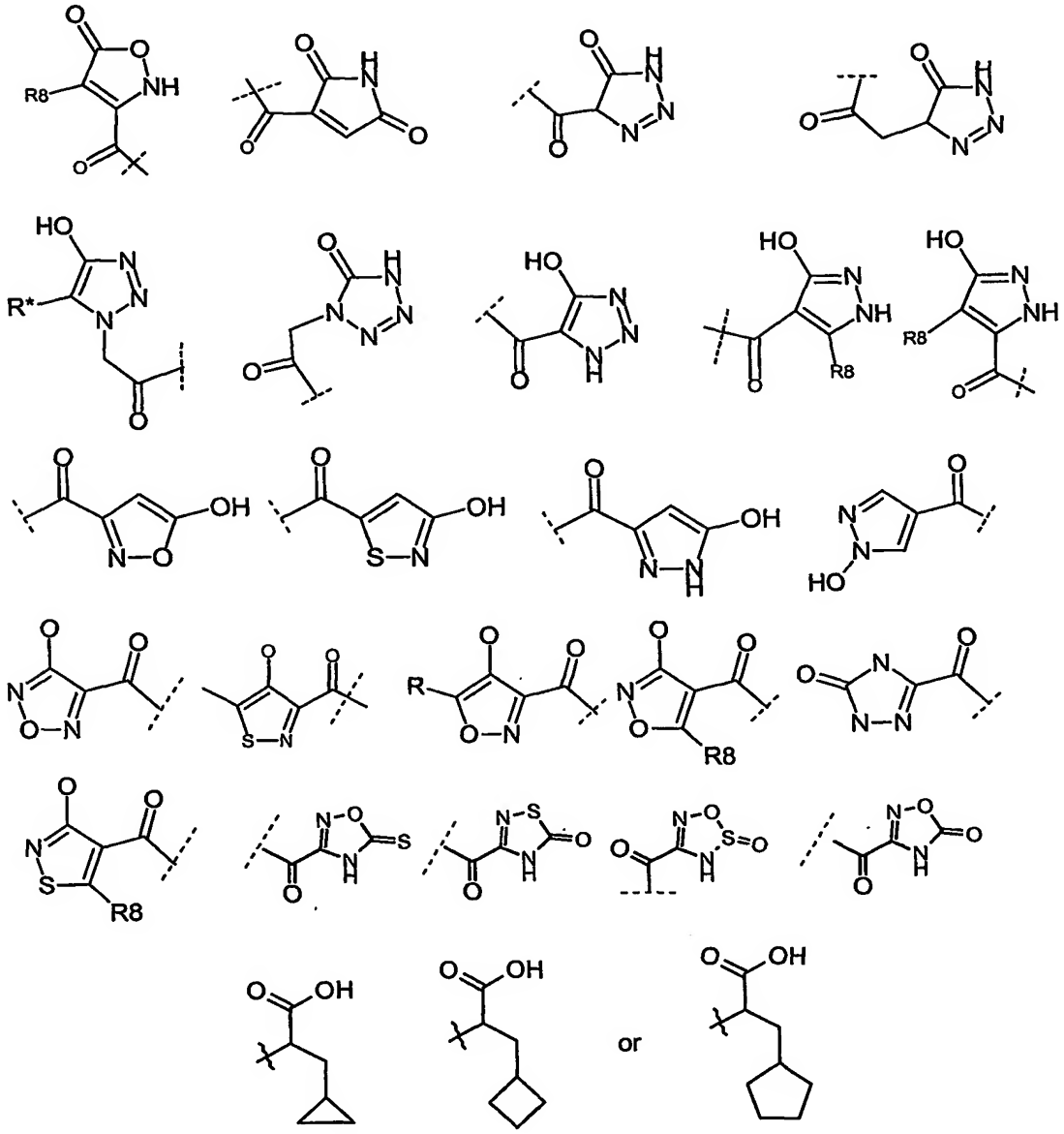
or

More suitably $-(Y)_m-R^3$ is









In one embodiment m is 1, Y is –C(O)-, and R^3 is aryl, heteroaryl, alkyl, or cycloalkyl, each optionally substituted.

In one embodiment m is 1, Y is –(C=N-CN)-O-, and R^3 is optionally substituted aryl, optionally substituted alkyl, optionally substituted cycloalkyl, optionally substituted heteroaryl, or optionally substituted heterocyclyl.

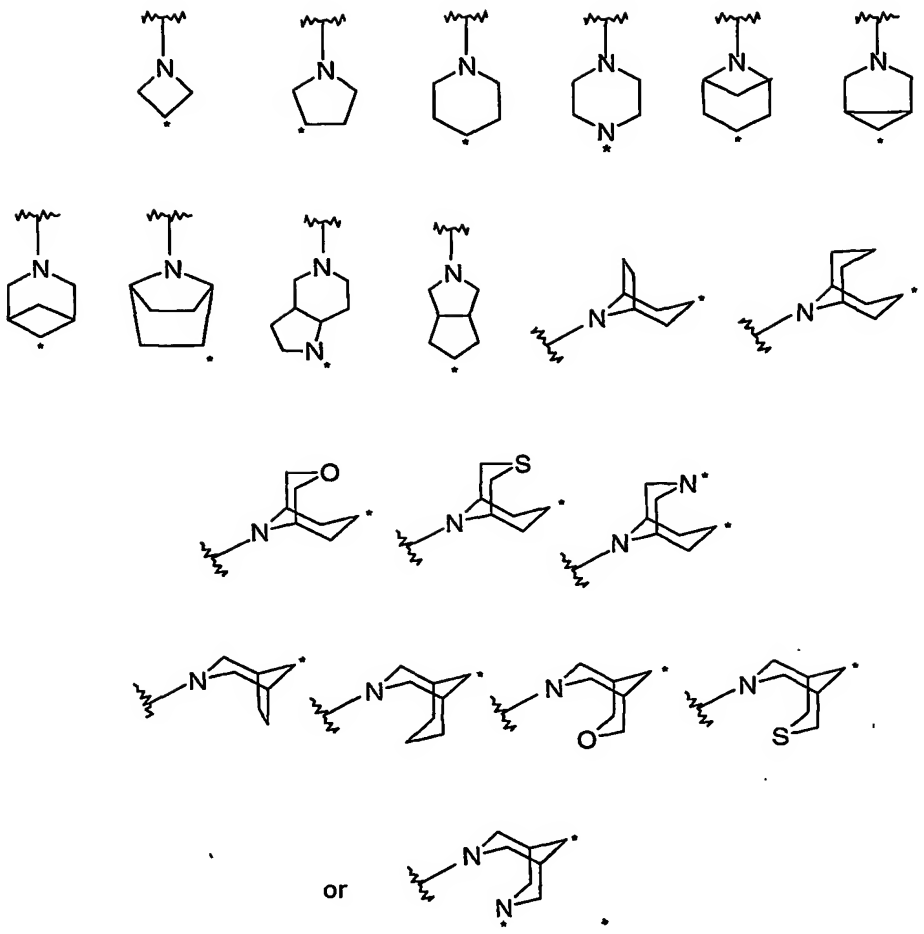
In one embodiment m is 1, Y is $-(CH_2)-$, and R^3 is optionally substituted aryl.

In one embodiment m is 1, Y is –C(O)O-, and R^3 is optionally substituted alkyl or optionally substituted aryl.

In one embodiment m is 0 and R^3 is optionally substituted heteroaryl or optionally substituted heterocyclyl.

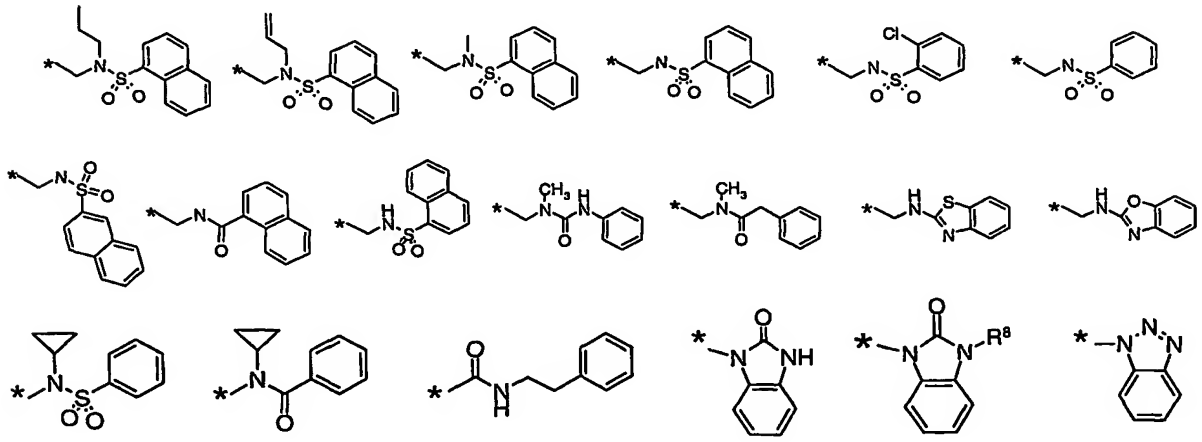
In one embodiment X is $–(CH_2)-$, $–(CH_2-CH_2)-$, or $–(CH_2-CH_2-CH_2)-$. Further X is optionally substituted by one or more halogen or oxo. Still further X is disubstituted with halogen. Still further X is disubstituted with fluoro. Specifically X may be $–(CF_2-CH_2)-$. Further X optionally has 1-3 heteroatoms selected from oxygen, phosphorus, sulfur, or nitrogen.

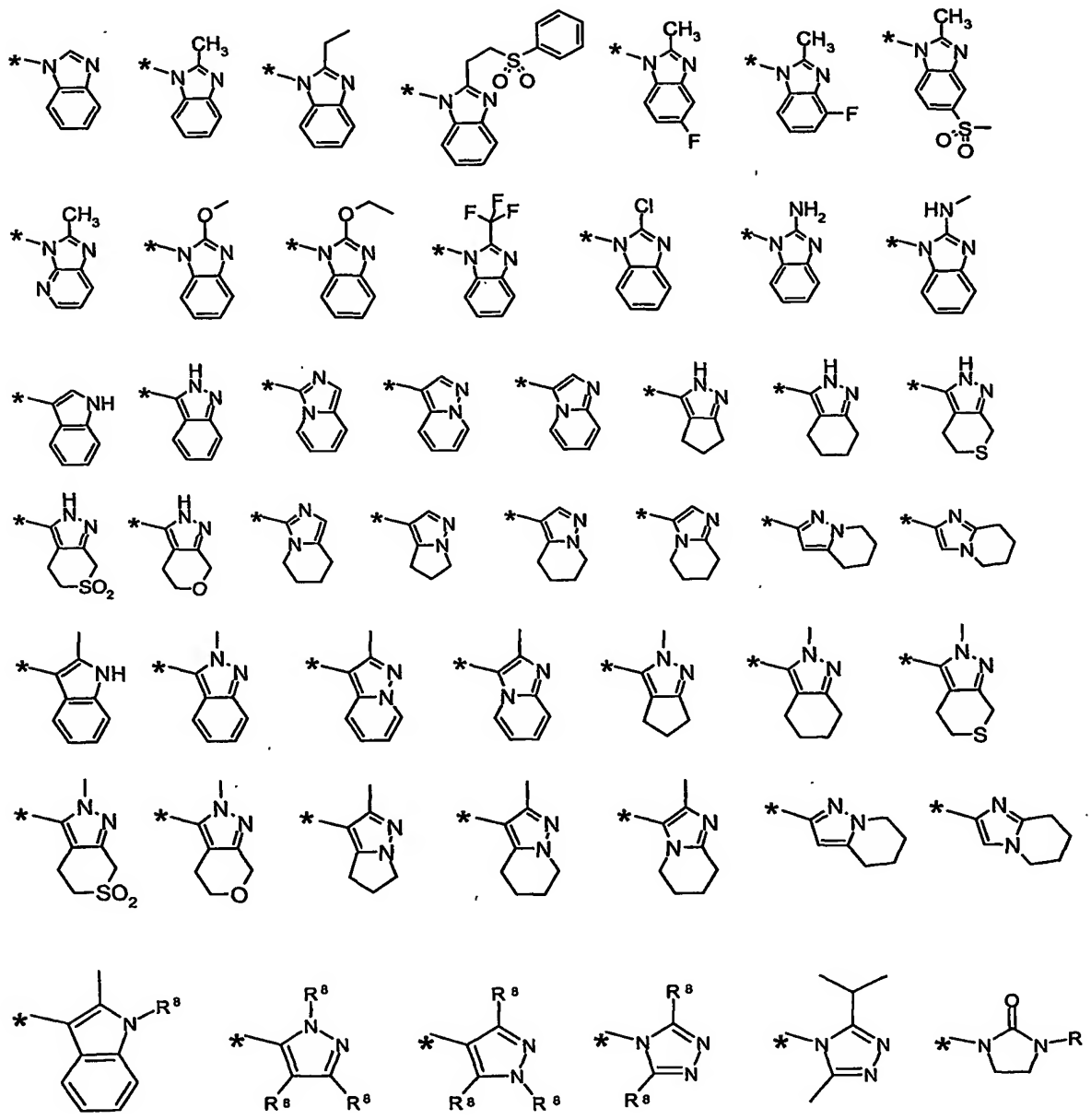
In one embodiment the A ring is selected from the following, where the asterisk (*) indicates the preferred, but not limiting, point(s) of substitution:

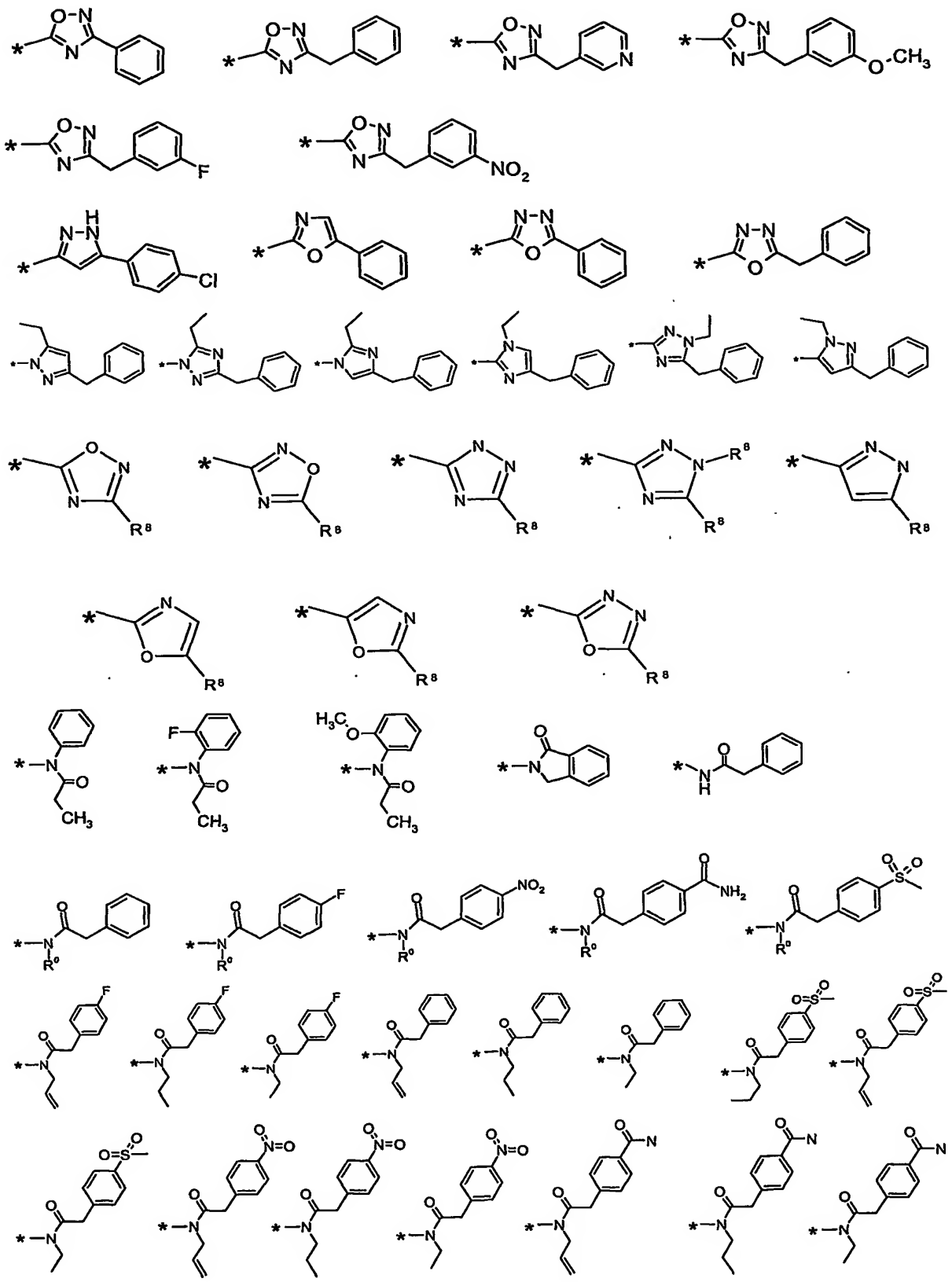


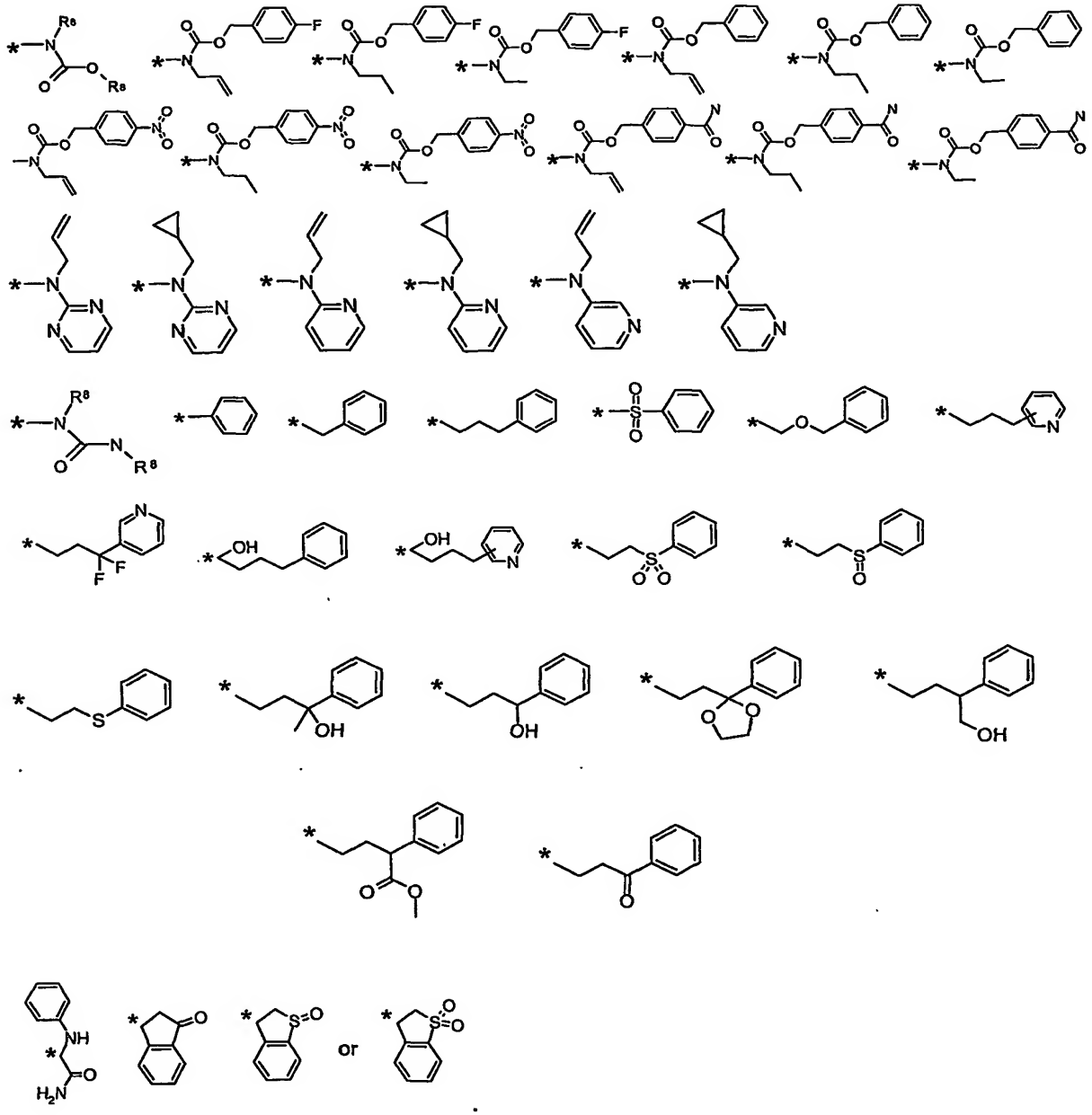
or

Suitably each R^2, with the asterisk (*) indicating a preferred, but not limiting, point of substitution from Ring A, independently is selected from

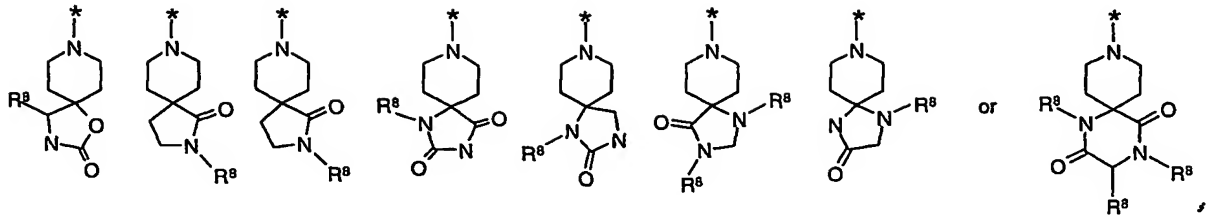




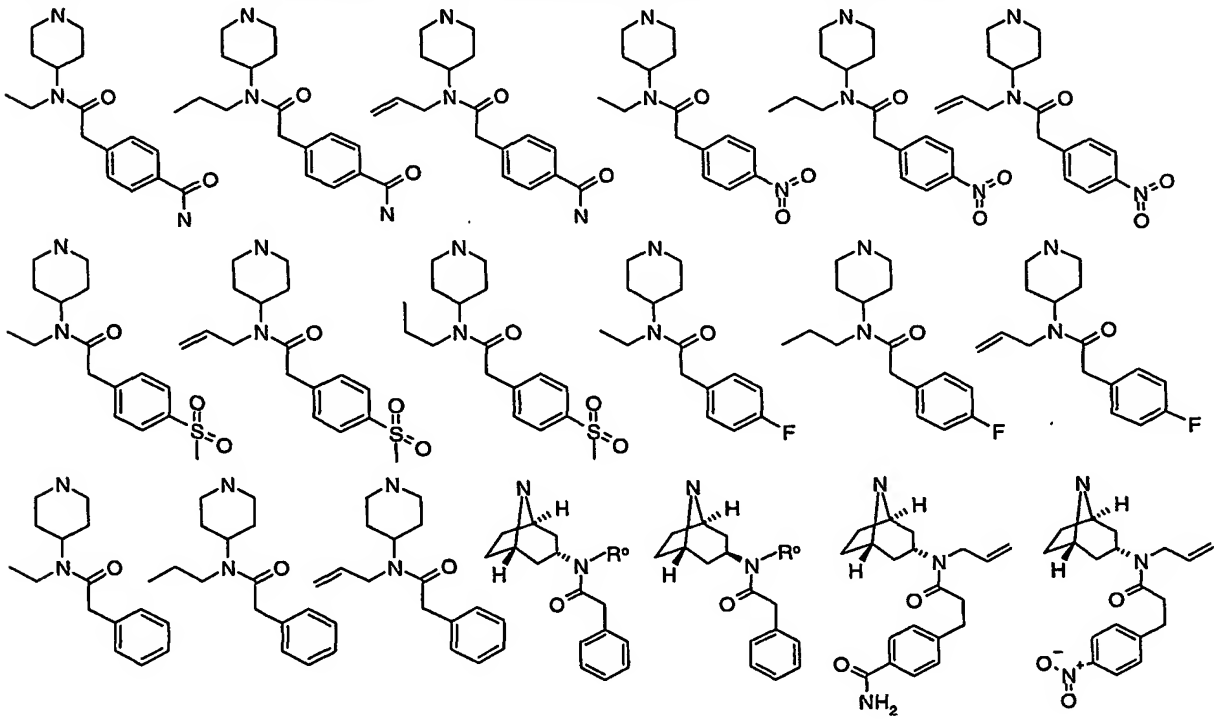


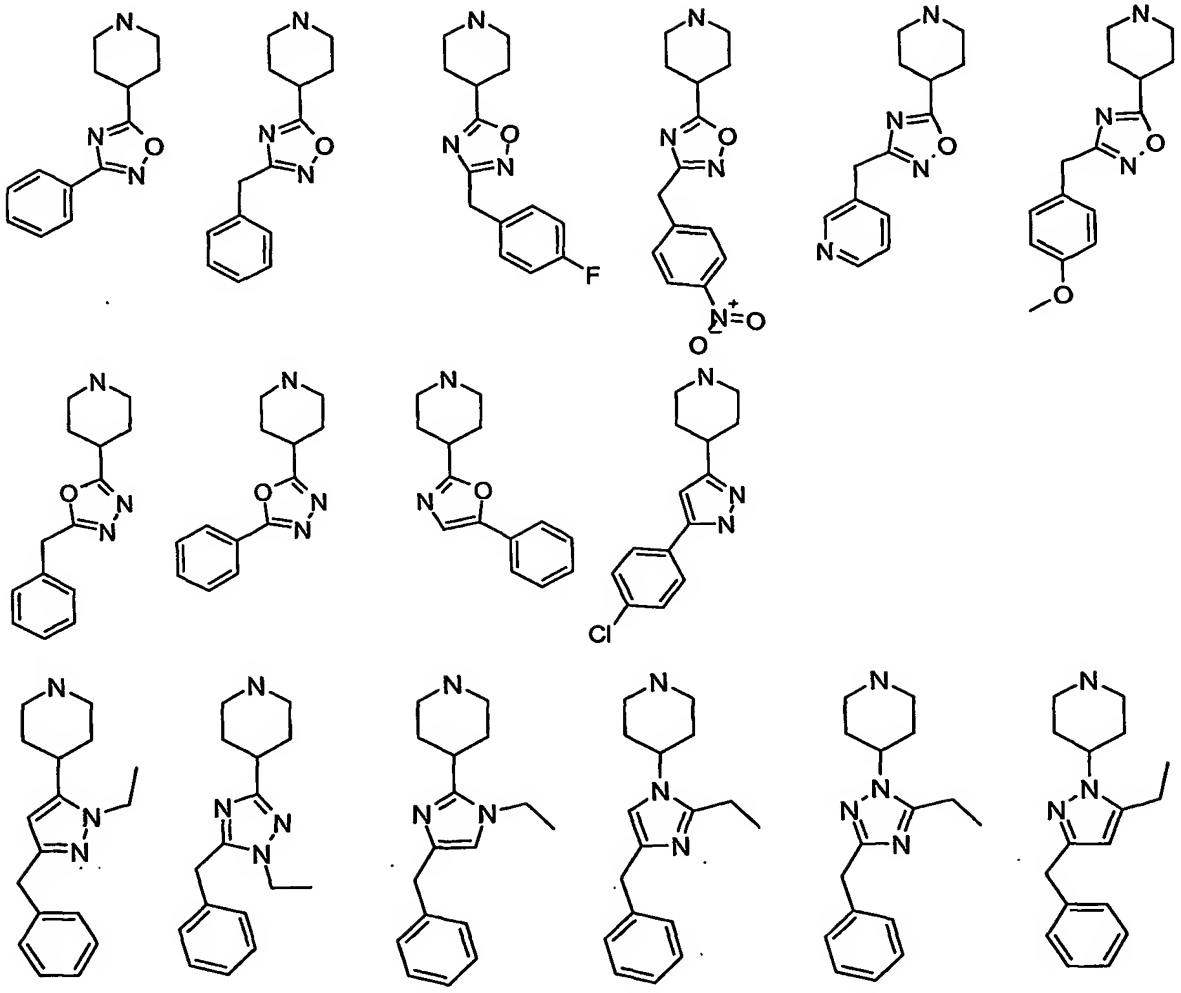


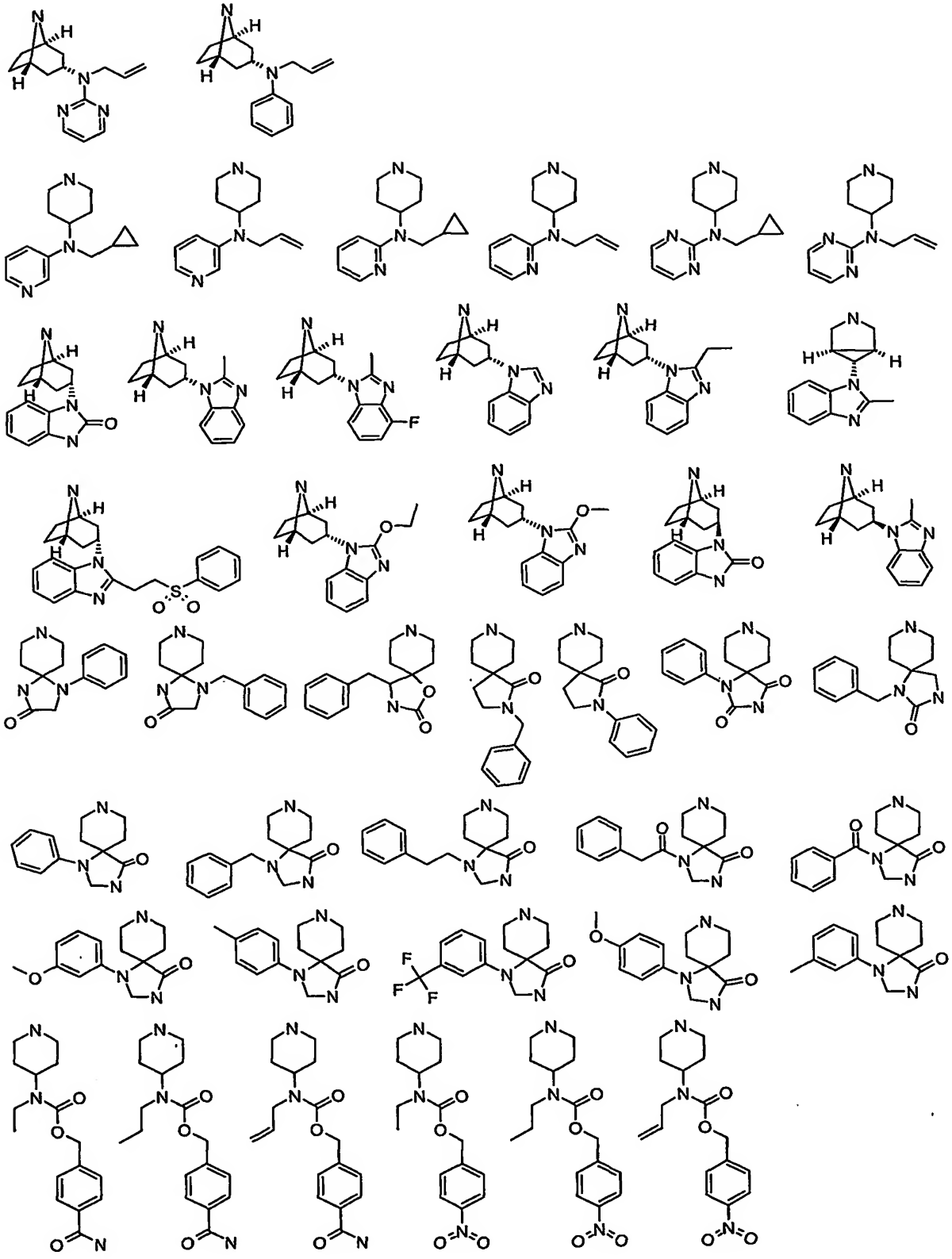
In one embodiment the ring A, with two geminal R^2s, is selected from:

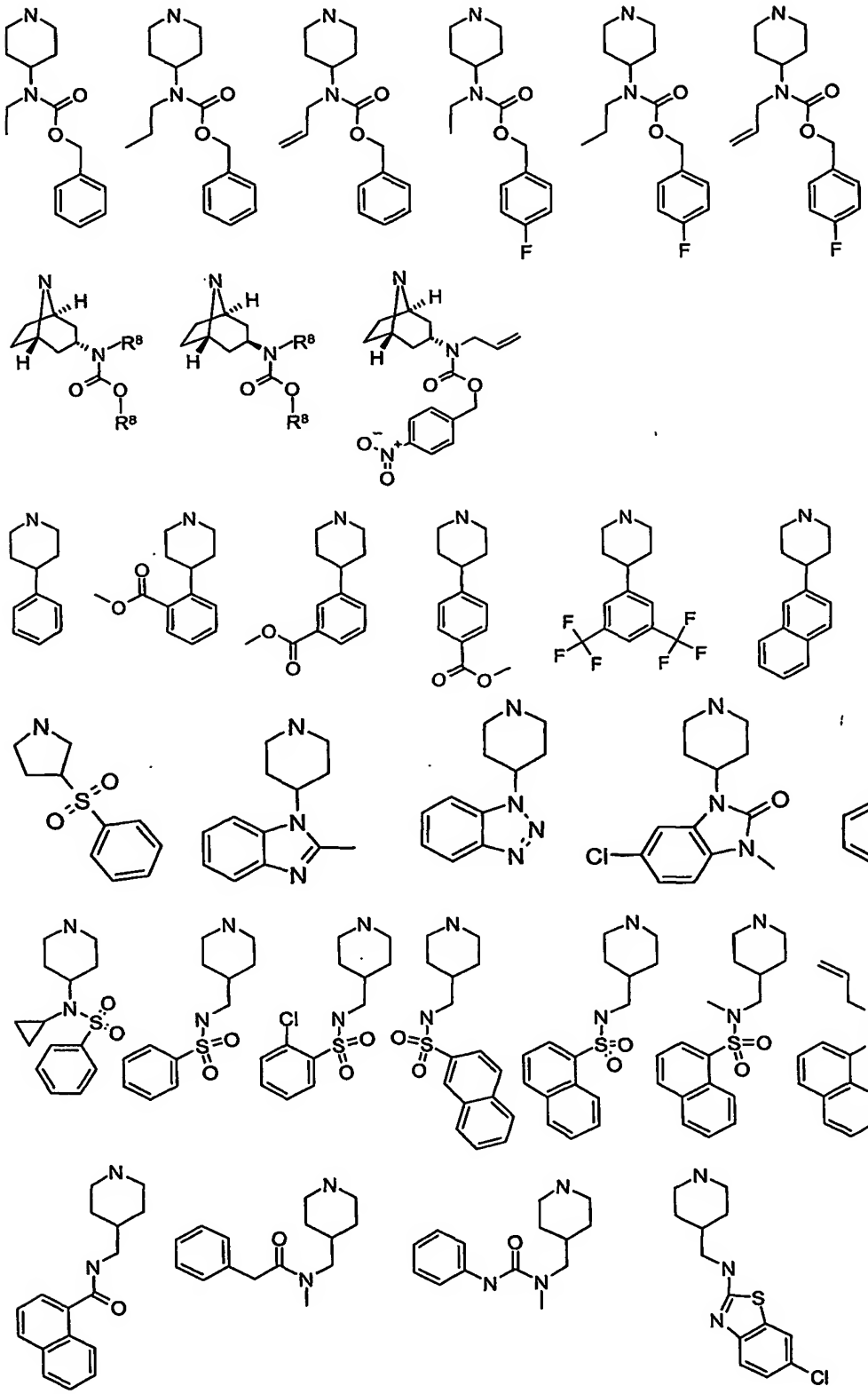


Suitably the A ring is tropane or piperidine, either optionally substituted with one or more R^2. Preferrably, A--R^2 is comprised of one of the following:









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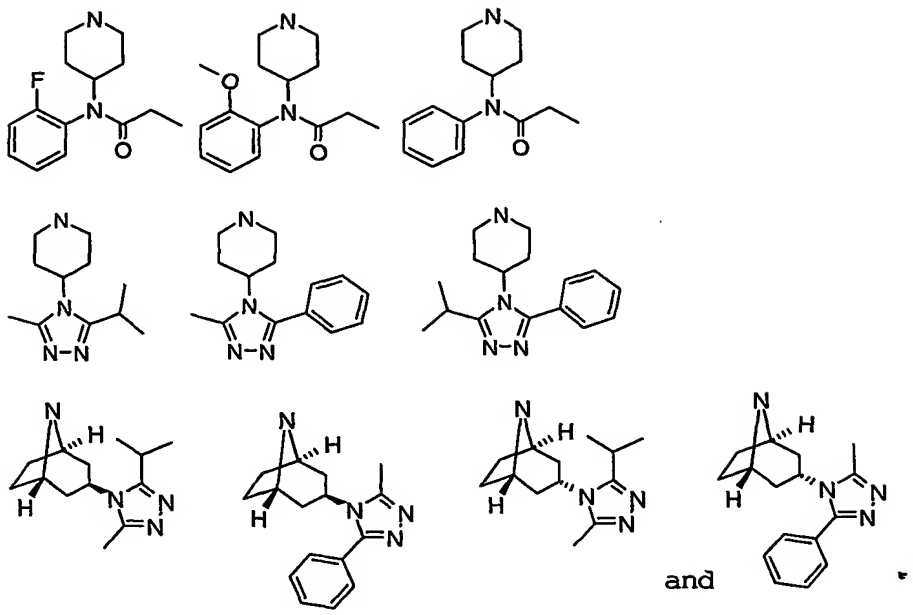
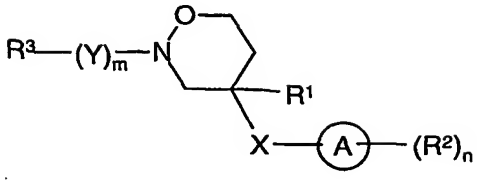
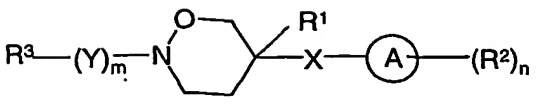


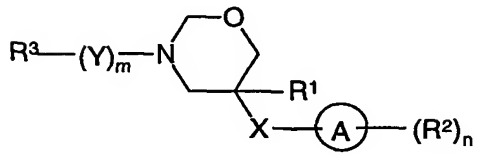
and

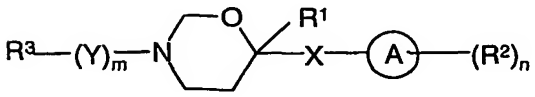
In one embodiment the A ring contains at least one additional nitrogen atom and said A ring optionally is N-substituted. Suitably the A ring is N-substituted with $-(CH_2)_a-(V_b-R+)$.

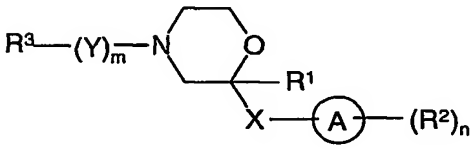
One aspect of the invention includes the compounds of claim 1 wherein ring B is as follows:

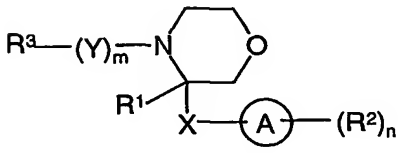




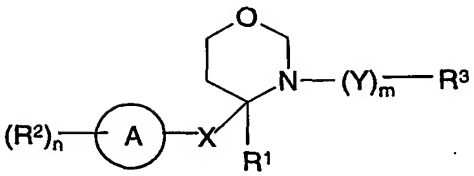








or



An additional aspect of the invention includes a method of treatment including prevention of a viral infection in a mammal comprising administering to said mammal an antiviral effective amount of a compound of the present invention. Preferably the viral infection is an HIV infection.

An additional aspect of the invention includes a method of treatment including prevention of a bacterial infection in a mammal comprising administering to said mammal an effective amount of a compound of the present invention. Preferably the bacterium is *Yersinia pestis*.

An additional aspect of the invention includes a method of treatment including prevention of multiple sclerosis, rheumatoid arthritis, autoimmune diabetes, chronic implant rejection, asthma, rheumatoid arthritis, Crohns Disease, inflammatory bowel disease, chronic inflammatory disease, glomerular disease, nephrotoxic serum

nephritis, kidney disease, Alzheimer's Disease , autoimmune encephalomyelitis, arterial thrombosis, allergic rhinitis, arteriosclerosis, Sjogren's syndrome (dermatomyositis), systemic lupus erythematosus, graft rejection, cancers with leukocyte infiltration of the skin or organs, infectious disorders including bubonic and pneumonic plague, human papilloma virus infection, prostate cancer, wound healing, amyotrophic lateral sclerosis and immune mediated disorders in a mammal comprising administering to said mammal a pharmceutically effective amount of a compound of the present invention.

An additional aspect of the invention includes a compound of the present invention for use in medical therapy.

An additional aspect of the invention includes the use of a compound of the present invention in the manufacture of a medicament for the treatment including prophylaxis of a viral infection. Preferably the viral infection is a HIV infection.

An additional aspect of the invention includes the use of a compound of the present invention in the manufacture of a medicament for the treatment including prophylaxis of a bacterial infection. Preferably the bacterium is *Yersinia pestis*.

An additional aspect of the invention includes the use of a compound of the present invention in the manufacture of a medicament for the treatment including prophylaxis of multiple sclerosis, rheumatoid arthritis, autoimmune diabetes, chronic implant rejection, asthma, rheumatoid arthritis, Crohns Disease, inflammatory bowel disease, chronic inflammatory disease, glomerular disease, nephrotoxic serum nephritis, kidney disease, Alzheimer's Disease , autoimmune encephalomyelitis, arterial thrombosis, allergic rhinitis, arteriosclerosis, Sjogren's syndrome (dermatomyositis), systemic lupus erythematosus, graft rejection, cancers with leukocyte infiltration of the skin or organs, infectious disorders including bubonic and pneumonic plague, human papilloma virus infection, prostate cancer, wound healing, amyotrophic lateral sclerosis and immune mediated disorders.

An additional aspect of the invention includes a pharmaceutical composition comprising a pharmaceutically effective amount of a compound of the present

invention together with a pharmaceutically acceptable carrier. Preferably the pharmaceutical composition is in the form of a tablet, capsule, or liquid.

An additional aspect of the invention includes a method of treatment including prevention of a viral infection in a mammal comprising administering to said mammal a composition comprising a compound of the present invention and another therapeutic agent. Preferably the composition further includes another therapeutic agent selected from the group consisting of (1-alpha, 2-beta, 3-alpha)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine [(-)BHCG, SQ-34514, lobucavir], 9-[(2R,3R,4S)-3,4-bis(hydroxymethyl)-2-oxetanosyl]adenine (oxetanocin-G), acyclic nucleosides, acyclovir, valaciclovir, famciclovir, ganciclovir, penciclovir, acyclic nucleoside phosphonates, (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine (HPMPC), [[[2-(6-amino-9H-purin-9-yl)ethoxy]methyl]phosphinylidene]bis(oxymethylene)-2,2-dimethylpropanoic acid (bis-POM PMEA, adefovir dipivoxil), [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]phosphonic acid (tenofovir), (R)-[[2-(6-Amino-9H-purin-9-yl)-1-methylethoxy]methyl]phosphonic acid bis-(isopropoxycarbonyloxymethyl)ester (bis-POC-PMPA), ribonucleotide reductase inhibitors, 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl) thiocarbonohydrazone and hydroxyurea, nucleoside reverse transcriptase inhibitors, 3'-azido-3'-deoxythymidine (AZT, zidovudine), 2',3'-dideoxycytidine (ddC, zalcitabine), 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine (ddI, didanosine), 2',3'-didehydrothymidine (d4T, stavudine), (-)-beta-D-2,6-diaminopurine dioxolane (DAPD), 3'-azido-2',3'-dideoxythymidine-5'-H-phosphophonate (phosphonovir), 2'-deoxy-5-iodo-uridine (idoxuridine), (-)-cis-1-(2-hydroxymethyl)-1,3-oxathiolane 5-yl)-cytosine (lamivudine), cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine (FTC), 3'-deoxy-3'-fluorothymidine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, (-)-cis-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (abacavir), 9-[4-hydroxy-2-(hydroxymethyl)but-1-yl]-guanine (H2G), ABT-606 (2HM-H2G) ribavirin, protease inhibitors, indinavir, ritonavir, nelfinavir, amprenavir, saquinavir, fosamprenavir, (R)-N-tert-butyl-3-[(2S,3S)-2-hydroxy-3-N-[(R)-2-N-(isoquinolin-5-yloxyacetyl)amino-3-methylthiopropanoyl]amino-4-phenylbutanoyl]-5,5- dimethyl-1,3-thiazolidine-4-carboxamide (KNI-272), 4R-(4alpha,5alpha,6beta)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one dimethanesulfonate (mozenavir), 3-[1-[3-[2-(5-trifluoromethylpyridinyl)-sulfonylamino]phenyl]propyl]-4- hydroxy-6alpha-

phenethyl-6beta-propyl-5,6-dihydro-2-pyranone (tipranavir), N'-[2(S)-Hydroxy-3(S)-[N-(methoxycarbonyl)-l-tert-leucylamino]-4- phenylbutyl-N alpha-(methoxycarbonyl)-N'-[4-(2-pyridyl)benzyl]-L- tert-leucylhydrazide (BMS-232632), 3-(2(S)-Hydroxy-3(S)-(3-hydroxy-2-methylbenzamido)-4-phenylbutanoyl)-5,5-dimethyl-N-(2-methylbenzyl)thiazolidine-4(R)-carboxamide (AG-1776), N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenyl-methyl-4(S)-hydroxy-5-(1-(1-(4-benzo[b]furanylmethyl)-2(S)-N'-(tert-butylcarboxamido)piperazinyl)pentanamide (MK-944A), interferons, α-interferon, renal excretion inhibitors, probenecid, nucleoside transport inhibitors, dipyridamole, pentoxifylline, N-acetylcysteine (NAC), Procysteine, α -trichosanthin, phosphonoformic acid, immunomodulators, interleukin II, thymosin, granulocyte macrophage colony stimulating factors, erythropoetin, soluble CD_4 and genetically engineered derivatives thereof, non-nucleoside reverse transcriptase inhibitors (NNRTIs), nevirapine (BI-RG-587), alpha-((2-acetyl-5-methylphenyl)amino)-2,6-dichloro-benzeneacetamide (loviride), 1-[3-(isopropylamino)-2-pyridyl]-4-[5-(methanesulfonamido)-1H-indol-2-ylcarbonyl]piperazine monomethanesulfonate (delavirdine), (10R, 11S, 12S)-12-hydroxy-6, 6, 10, 11-tetramethyl-4-propyl-11,12-dihydro-2H, 6H, 10H-benzo(1, 2-b:3, 4-b':5, 6-b")tripyran-2-one ((+) calanolide A), (4S)-6-Chloro-4-[1E)-cyclopropylethenyl)-3,4- dihydro-4-(trifluoromethyl)-2(1H)-quinazolinone (DPC-083), (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one (efavirenz, DMP 266), 1-(ethoxymethyl)-5-(1-methylethyl)-6-(phenylmethyl)-2,4(1H,3H)-pyrimidinedione (MKC-442), and 5-(3,5-dichlorophenyl)thio-4-isopropyl-1-(4-pyridyl)methyl-1H-imidazol-2-ylmethyl carbamate (capravirine), glycoprotein 120 antagonists, PRO-2000, PRO-542, 1,4-bis[3-[(2, 4- dichlorophenyl)carbonylamino]-2-oxo-5,8-disodiumsulfanyl]naphthalyl-2, 5-dimethoxyphenyl-1, 4-dihydrazone (FP-21399), cytokine antagonists, reticulose (Product-R), 1,1'-azobis-formamide (ADA), 1,11-(1,4-phenylenebis(methylene))bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride (AMD-3100), integrase inhibitors, and fusion inhibitors.

An additional aspect of the invention includes a method of treatment including prevention of a viral infection in a mammal comprising administering to said mammal a composition comprising a compound of the present invention and ritonavir.

DETAILED DESCRIPTION OF THE INVENTION

The phrase "optionally substituted" is used interchangeably with the phrase "substituted or unsubstituted" or with the term "(un)substituted." Unless otherwise indicated, an optionally substituted group may have a substituent at each substitutable position of the group, and each substitution is independent of the other.

The term "alkyl", alone or in combination with any other term, refers to a straight-chain or branched-chain saturated aliphatic hydrocarbon radical containing the specified number of carbon atoms. Examples of alkyl radicals include, but are not limited to, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, isoamyl, n-hexyl and the like.

The term "cycloalkyl", "carbocylyl", "carbocyclic", or "carbocycle", or "carbocyclo", alone or in combination with any other term, refers to a monocyclic or polycyclic non-aromatic hydrocarbon ring radical having three to twenty carbon atoms, preferably from three to twelve carbon atoms, and more preferably from three to ten carbon atoms. If polycyclic, each ring in a carbocylyl radical is non-aromatic unless otherwise indicated. A carbocylyl radical is either completely saturated or contains one or more units of unsaturation but is not aromatic. The unsaturation, if present, may occur in any point in the ring that may result in any chemically stable configuration. The term "cycloalkyl", "carbocylyl", "carbocyclic", or "carbocycle", or "carbocyclo" also includes hydrocarbon rings that are fused to one or more aromatic rings, such as in tetrahydronaphthyl, where the radical or point of attachment is on the non-aromatic ring.

Unless otherwise indicated, the term "cycloalkyl", "carbocylyl", "carbocyclic", or "carbocycle", or "carbocyclo" also includes each possible positional isomer of a non-aromatic hydrocarbon radical, such as in 1-decahydronaphthyl, 2-decahydronaphthyl, 1-tetrahydronaphthyl and 2-tetrahydronaphthyl. Examples of suitable cycloalkyl groups include, but are not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexenyl, decahydronaphthyl, tetrahydronaphthyl and the like.

The term "alkenyl," alone or in combination with any other term, refers to a straight-chain or branched-chain alkyl group with at least one carbon-carbon double bond. Examples of alkenyl radicals include, but are not limited to, ethenyl, propenyl, isopropenyl, butenyl, isobutenyl, pentenyl, hexenyl, hexadienyl and the like.

The term "alkynyl" refers to hydrocarbon groups of either a straight or branched configuration with one or more carbon-carbon triple bonds which may occur in any stable point along the chain, such as ethynyl, propynyl, butynyl, pentynyl, and the like.

The term "alkoxy" refers to an alkyl ether radical, wherein the term "alkyl" is defined above. Examples of suitable alkyl ether radicals include, but are not limited to, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, isobutoxy, sec-butoxy, tert-butoxy and the like.

The term "aryl", alone or in combination with any other term, refers to an aromatic monocyclic or polycyclic hydrocarbon ring radical containing five to twenty carbon atoms, preferably from six to fourteen carbon atoms, and more preferably from six to ten carbon atoms. Also included within the scope of the term "aryl", as it is used herein, is a group in which an aromatic hydrocarbon ring is fused to one or more non-aromatic carbocyclic or heteroatom-containing rings, such as in an indanyl, phenanthridinyl or tetrahydronaphthyl, where the radical or point of attachment is on the aromatic hydrocarbon ring.

Unless otherwise indicated, the term "aryl" also includes each possible positional isomer of an aromatic hydrocarbon radical, such as in 1-naphthyl, 2-naphthyl, 5-tetrahydronaphthyl, 6-tetrahydronaphthyl, 1-phenanthridinyl, 2-phenanthridinyl, 3-phenanthridinyl, 4-phenanthridinyl, 7-phenanthridinyl, 8-phenanthridinyl, 9-phenanthridinyl and 10-phenanthridinyl. Examples of aryl radicals include, but are not limited to, phenyl, naphthyl, indenyl, azulenyl, fluorenyl, anthracenyl, phenanthrenyl, tetrahydronaphthyl, indanyl, phenanthridinyl and the like.

The term "aralkyl" further refers to groups of $-R_aR_b$, where R_a is an alkylene as defined herein and R_b is an aryl as defined herein.

The term "heterocycle", "heterocyclic", and "heterocyclyl", alone or in combination with any other term, refers to a non-aromatic monocyclic or polycyclic ring radical containing three to twenty carbon atoms, preferably three to seven carbon atoms if monocyclic and eight to eleven carbon atoms if bicyclic, and in which one or more ring carbons, preferably one to four, are each replaced by a heteroatom such as N, O, and S. If polycyclic, each ring in a heterocyclyl radical is non-aromatic unless otherwise indicated. A heterocyclic ring may be fully saturated or may contain one or more units of unsaturation but is not aromatic. The unsaturation, if present, may occur in any point in the ring that may result in any chemically stable configuration. The heterocyclic ring may be attached at a carbon or heteroatom that results in the creation of a stable structure. Preferred heterocycles include 5-7 membered monocyclic heterocycles and 8-10 membered bicyclic heterocycles.

Also included within the scope of the term "heterocycle", "heterocyclic", or "heterocyclyl" is a group in which a non-aromatic heteroatom-containing ring is fused to one or more aromatic rings, such as in an indolinyl, chromanyl, phenanthridinyl or tetrahydroquinolinyl, where the radical or point of attachment is on the non-aromatic heteroatom-containing ring. Unless otherwise indicated, the term "heterocycle", "heterocyclic", or "heterocyclyl" also includes each possible positional isomer of a heterocyclic radical, such as in 1-decahydroquinoline, 2-decahydroquinoline, 3-decahydroquinoline, 4-decahydroquinoline, 5-decahydroquinoline, 6-decahydroquinoline, 7-decahydroquinoline, 7-decahydroquinoline, 8-decahydroquinoline, 4a-decahydroquinoline, 8a-decahydroquinoline, 1-indolinyl, 2-indolinyl, 3-indolinyl, 1-tetrahydroquinoline, 2-tetrahydroquinoline, 3-tetrahydroquinoline and 4-tetrahydroquinoline. The term "heterocyclylalkyl" refers to an alkyl group substituted by a heterocyclyl.

Examples of heterocyclic groups include, but are not limited to, imidazolinyl, 2,3-dihydro-1H-imidazolyl, imidazolidinyl, indazolinolyl, perhydropyridazyl, pyrrolinyl, pyrrolidinyl, 4H-pyrazolyl, piperidinyl, pyranyl, pyrazolinyl, piperazinyl, morpholinyl, thiamorpholinyl, thiazolidinyl, thiamorpholinyl, oxopiperidinyl, oxopyrrolidinyl, azepinyl, tetrahydrofuranyl, oxoazepinyl, tetrahydropyranyl, thiazolyl, dioxolyl, dioxinyl, oxathiolyl, benzodioxolyl, dithiolyl, dithiolanyl, tetrahydrothiophenyl, sulfolanyl, dioxanyl, dioxolanyl, tetahydrofurodihydrofuranyl, dihydropyranyl, tetrahydropyranodihydrofuranyl, tetradyrofurofuranyl, tetrahydropyranofuranyl,

diazolonyl, phthalimidinyl, benzoxanyl, benzopyrrolidinyl, benzopiperidinyl, benzoxolanyl, benzothiolanyl and benzothianyl.

The term "heteroaryl", alone or in combination with any other term, refers to an aromatic monocyclic or polycyclic ring radical containing five to twenty carbon atoms, preferably five to ten carbon atoms, in which one or more ring carbons, preferably one to four, are each replaced by a heteroatom such as N, O, and S. Preferred heteroaryl groups include 5-6 membered monocyclic heteroaryls and 8-10 membered bicyclic heteroaryls.

Also included within the scope of the term "heteroaryl" is a group in which a heteroaromatic ring is fused to one or more aromatic or non-aromatic rings where the radical or point of attachment is on the heteroaromatic ring. Examples include, but are not limited to, pyrido[3,4-d]pyrimidinyl, 7,8-dihydro-pyrido[3,4-d]pyrimidine and 5,6,7,8-tetrahydro-pyrido[3,4-d]pyrimidine. Unless otherwise indicated, the term "heteroaryl" also includes each possible positional isomer of a heteroaryl radical, such as in 2-pyrido[3,4-d]pyrimidinyl and 4-pyrido[3,4-d]pyrimidinyl.

Examples of heteroaryl groups include, but are not limited to, imidazolyl, quinolyl, isoquinolyl, indolyl, indazolyl, pyridazyl, pyridyl, pyrrolyl, pyrazolyl, pyrazinyl, quinoxalyl, pyrimidinyl, pyridazinyl, furyl, thienyl, triazolyl, thiazolyl, carbazolyl, carbolinyl, tetrazolyl, benzofuranyl, oxazolyl, benzoxazolyl, isoxozolyl, isothiazolyl, thiadiazolyl, furazanyl, oxadiazolyl, benzimidazolyl, benzothienyl, quinolinyl, benzotriazolyl, benzothiazolyl, isoquinolinyl, isoindolyl, acridinyl and benzoisoxazolyl.

The term "heteroaralkyl" further refers to groups of $-R_aR_b$, where R_a is an alkylene as defined herein and R_b is a heteroaryl as defined herein.

The term "heteroatom" means nitrogen, oxygen, phosphorus, or sulfur and includes any oxidized forms thereof, including as non-limiting examples oxidized forms of nitrogen such as N(O) $\{N^+-O^-\}$, oxidized forms of sulfur such as S(O) and $S(O)_2$, and the quaternized form of any basic nitrogen.

The term "halogen" refers to fluorine, chlorine, bromine or iodine.

The term "pharmaceutically effective amount" refers to an amount of a compound of the invention that is effective in treating a CCR5-related disease, for example a virus infection, for example an HIV infection, in a patient either as monotherapy or in combination with other agents. The term "treatment" as used herein refers to the alleviation of symptoms of a particular disorder in a patient, or the improvement of an ascertainable measurement associated with a particular disorder, and may include the suppression of symptom recurrence in an asymptomatic patient such as a patient in whom a viral infection has become latent. The term "prophylaxis" refers to preventing a disease or condition or preventing the occurrence of symptoms of such a disease or condition, in a patient. As used herein, the term "patient" refers to a mammal, including a human.

The term "pharmaceutically acceptable carrier" refers to a carrier that may be administered to a patient, together with a compound of this invention, and which does not destroy the pharmacological activity thereof and is nontoxic when administered in doses sufficient to deliver a therapeutic amount of the therapeutic agent.

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ester, salt of an ester, or other derivative of a compound of this invention which, upon administration to a recipient, is capable of providing (directly or indirectly) a compound of this invention or an inhibitorily active metabolite or residue thereof. Particularly favored derivatives and prodrugs are those that increase the bioavailability of the compounds of this invention when such compounds are administered to a mammal (e.g., by allowing an orally administered compound to be more readily absorbed into the blood) or which enhance delivery of the parent compound to a biological compartment (e.g., the brain or lymphatic system) relative to the parent species.

Pharmaceutically acceptable salts of the compounds according to the invention include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulfuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicyclic, succinic, toluene-p-sulfonic, tartaric, acetic, citric, methanesulfonic, ethanesulfonic, formic, benzoic, malonic, naphthalene-2-sulfonic and benzenesulfonic acids. Other acids, such as oxalic, while not in themselves

pharmaceutically acceptable, may be employed in the preparation of salts useful as intermediates in obtaining the compounds of the invention and their pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g., magnesium), ammonium, NW_4^+ (wherein W is C_{1-4} alkyl) and other amine salts. Physiologically acceptable salts of a hydrogen atom or an amino group include salts of organic carboxylic acids such as acetic, lactic, tartaric, malic, isethionic, lactobionic and succinic acids; organic sulfonic acids such as methanesulfonic, ethanesulfonic, benzenesulfonic and p-toluenesulfonic acids and inorganic acids such as hydrochloric, sulfuric, phosphoric and sulfamic acids. Physiologically acceptable salts of a compound with a hydroxy group include the anion of said compound in combination with a suitable cation such as Na^+, NH_4^+, and NW_4^+ (wherein W is a C_{1-4}alkyl group).

Any reference to any of the above compounds also includes a reference to a pharmaceutically acceptable salt thereof.

Salts of the compounds of the present invention may be made by methods known to a person skilled in the art. For example, treatment of a compound of the present invention with an appropriate base or acid in an appropriate solvent will yield the corresponding salt.

Esters of the compounds of the present invention are independently selected from the following groups: (1) carboxylic acid esters obtained by esterification of the hydroxy groups, in which the non-carbonyl moiety of the carboxylic acid portion of the ester grouping is selected from straight or branched chain alkyl (for example, acetyl, n-propyl, t-butyl, or n-butyl), alkoxyalkyl (for example, methoxymethyl), aralkyl (for example, benzyl), aryloxyalkyl (for example, phenoxymethyl), aryl (for example, phenyl optionally substituted by, for example, halogen, C_{1-4}alkyl, or C_{1-4}alkoxy or amino); (2) sulfonate esters, such as alkyl- or aralkylsulfonyl (for example, methanesulfonyl); (3) amino acid esters (for example, L-valyl or L-isoleucyl); (4) phosphonate esters and (5) mono-, di- or triphosphate esters. The phosphate esters may be further esterified by, for example, a C_{1-20} alcohol or reactive derivative thereof, or by a 2,3-di (C_{6-24})acyl glycerol.

In such esters, unless otherwise specified, any alkyl moiety present advantageously contains from 1 to 18 carbon atoms, particularly from 1 to 6 carbon atoms, more particularly from 1 to 4 carbon atoms, Any cycloalkyl moiety present in such esters advantageously contains from 3 to 6 carbon atoms. Any aryl moiety present in such esters advantageously comprises a phenyl group.

The compounds according to the invention contain one or more asymmetric carbon atoms and thus occur as racemates and racemic mixtures, single enantiomers, diastereomeric mixtures and individual diastereoisomers. All such isomeric forms of these compounds are expressly included in the present invention. Each stereogenic carbon may be of the R or S configuration. Although the specific compounds exemplified in this application may be depicted in a particular stereochemical configuration, compounds having either the opposite stereochemistry at any given chiral center or mixtures thereof are also envisioned.

Unless otherwise stated, structures depicted herein are also meant to include compounds which differ only in the presence of one or more isotopically enriched atoms. For example, compounds having the present structures except for the replacement of a hydrogen by a deuterium or tritium, or the replacement of a carbon by a ^{13}C- or ^{14}C-enriched carbon are also within the scope of this invention.

Certain compounds of this invention may exist in alternative tautomeric forms. All such tautomeric forms of the present compounds are within the scope of the invention. Unless otherwise indicated, the representation of either tautomer is meant to include the other.

The present invention features compounds according to the invention for use in medical therapy, for example for the treatment including prophylaxis of viral infections such as an HIV infections and associated conditions. Reference herein to treatment extends to prophylaxis as well as the treatment of established infections, symptoms, and associated clinical conditions such as AIDS related complex (ARC), Kaposi's sarcoma, and AIDS dementia.

The present invention features use of the compounds of the present invention in the manufacture of a medicament for the treatment including prophylaxis of a CCR5-related disease or condition, for example, a viral infection, for example, an HIV infection.

According to another aspect, the present invention provides a method for the treatment including prevention of the symptoms or effects of a viral infection in an infected animal, for example, a mammal including a human, which comprises treating said animal with a pharmaceutically effective amount of a compound according to the invention. According to one aspect of the invention, the viral infection is a retroviral infection, in particular an HIV infection. A further aspect of the invention includes a method for the treatment or prevention of the symptoms or effects of an HBV infection.

The compounds according to the invention may also be used in adjuvant therapy in the treatment of HIV infections or HIV-associated symptoms or effects, for example Kaposi's sarcoma.

The compounds of the present invention may also be used in the treatment including prevention of other CCR5-related diseases and conditions, including multiple sclerosis, rheumatoid arthritis, autoimmune diabetes, chronic implant rejection, asthma, rheumatoid arthritis, Crohns Disease, inflammatory bowel disease, chronic inflammatory disease, glomerular disease, nephrotoxic serum nephritis, kidney disease, Alzheimer's Disease , autoimmune encephalomyelitis, arterial thrombosis, allergic rhinitis, arteriosclerosis, Sjogren's syndrome (dermatomyositis), systemic lupus erythematosus, graft rejection, cancers with leukocyte infiltration of the skin or organs, human papilloma virus infection, prostate cancer, wound healing, amyotrophic lateral sclerosis, immune mediated disorders.

The present invention further provides a method for the treatment of a clinical condition in an animal, for example, a mammal including a human which clinical condition includes those which have been discussed hereinbefore, which comprises treating said animal with a pharmaceutically effective amount of a compound according to the invention. The present invention also includes a method for the treatment including prophylaxis of any of the aforementioned diseases or conditions.

In yet a further aspect, the present invention provides the use of a compound according to the invention in the manufacture of a medicament for the treatment including prophylaxis of any of the above mentioned viral infections or conditions.

The above compounds according to the invention and their pharmaceutically acceptable derivatives may be employed in combination with other therapeutic agents for the treatment of the above infections or conditions. Combination therapies according to the present invention comprise the administration of a compound of the present invention or a pharmaceutically acceptable derivative thereof and another pharmaceutically active agent. The active ingredient(s) and pharmaceutically active agents may be administered simultaneously in either the same or different pharmaceutical compositions or sequentially in any order. The amounts of the active ingredient(s) and pharmaceutically active agent(s) and the relative timings of administration will be selected in order to achieve the desired combined therapeutic effect.

Examples of such therapeutic agents include agents that are effective for the treatment of viral infections or associated conditions. Among these agents are (1-alpha, 2-beta, 3-alpha)-9-[2,3-bis(hydroxymethyl)cyclobutyl]guanine [(-)BHCG, SQ-34514, lobucavir], 9-[(2R,3R,4S)-3,4-bis(hydroxymethyl)-2-oxetanosyl]adenine (oxetanocin-G), acyclic nucleosides, for example acyclovir, valaciclovir, famciclovir, ganciclovir, and penciclovir, acyclic nucleoside phosphonates, for example (S)-1-(3-hydroxy-2-phosphonyl-methoxypropyl)cytosine (HPMPC), [[[2-(6-amino-9H-purin-9-yl)ethoxy]methyl]phosphinylidene]bis(oxymethylene)-2,2-dimethylpropanoic acid (bis-POM PMEA, adefovir dipivoxil), [[(1R)-2-(6-amino-9H-purin-9-yl)-1-methylethoxy]methyl]phosphonic acid (tenofovir), and (R)-[[2-(6-Amino-9H-purin-9-yl)-1-methylethoxy]methyl]phosphonic acid bis-(isopropoxycarbonyloxymethyl)ester (bis-POC-PMPA), ribonucleotide reductase inhibitors, for example 2-acetylpyridine 5-[(2-chloroanilino)thiocarbonyl) thiocarbonohydrazone and hydroxyurea, nucleoside reverse transcriptase inhibitors, for example 3'-azido-3'-deoxythymidine (AZT, zidovudine), 2',3'-dideoxycytidine (ddC, zalcitabine), 2',3'-dideoxyadenosine, 2',3'-dideoxyinosine (ddI, didanosine), 2',3'-didehydrothymidine (d4T, stavudine), (-)-beta-D-2,6-diaminopurine dioxolane (DAPD), 3'-azido-2',3'-dideoxythymidine-5'-H-phosphophonate (phosphonovir), 2'-deoxy-5-iodo-uridine (idoxuridine), (-)-cis-1-(2-

hydroxymethyl)-1,3-oxathiolane 5-yl)-cytosine (lamivudine), cis-1-(2-(hydroxymethyl)-1,3-oxathiolan-5-yl)-5-fluorocytosine (FTC), 3'-deoxy-3'-fluorothymidine, 5-chloro-2',3'-dideoxy-3'-fluorouridine, (-)-cis-4-[2-amino-6-(cyclopropylamino)-9H-purin-9-yl]-2-cyclopentene-1-methanol (abacavir), 9-[4-hydroxy-2-(hydroxymethyl)but-1-yl]-guanine (H2G), ABT-606 (2HM-H2G) and ribavirin, protease inhibitors, for example indinavir, ritonavir, nelfinavir, amprenavir, saquinavir, fosamprenavir, (R)-N-tert-butyl-3-[(2S,3S)-2-hydroxy-3-N-[(R)-2-N-(isoquinolin-5-yloxyacetyl)amino-3-methylthiopropanoyl]amino-4-phenylbutanoyl]-5,5-dimethyl-1,3-thiazolidine-4-carboxamide (KNI-272), 4R-(4alpha,5alpha,6beta)]-1,3-bis[(3-aminophenyl)methyl]hexahydro-5,6-dihydroxy-4,7-bis(phenylmethyl)-2H-1,3-diazepin-2-one dimethanesulfonate (mozenavir), 3-[1-[3-[2-(5-trifluoromethylpyridinyl)-sulfonylamino]phenyl]propyl]-4- hydroxy-6alpha-phenethyl-6beta-propyl-5,6-dihydro-2-pyranone (tipranavir), N'-[2(S)-Hydroxy-3(S)-[N-(methoxycarbonyl)-l-tert-leucylamino]-4- phenylbutyl-N ^{alpha}-(methoxycarbonyl)-N'-[4-(2-pyridyl)benzyl]-L- tert-leucylhydrazide (BMS-232632), 3-(2(S)-Hydroxy-3(S)-(3-hydroxy-2-methylbenzamido)-4-phenylbutanoyl)-5,5-dimethyl-N-(2-methylbenzyl)thiazolidine-4(R)-carboxamide (AG-1776), N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenyl-methyl-4(S)-hydroxy-5-(1-(1-(4-benzo[b]furanylmethyl)-2(S)-N'-(tert-butylcarboxamido)piperazinyl)pentanamide (MK-944A), interferons such as α-interferon, renal excretion inhibitors such as probenecid, nucleoside transport inhibitors such as dipyridamole; pentoxifylline, N-acetylcysteine (NAC), Procysteine, α -trichosanthin, phosphonoformic acid, as well as immunomodulators such as interleukin II or thymosin, granulocyte macrophage colony stimulating factors, erythropoetin, soluble CD_4 and genetically engineered derivatives thereof, non-nucleoside reverse transcriptase inhibitors (NNRTIs), for example nevirapine (BI-RG-587), alpha-((2-acetyl-5-methylphenyl)amino)-2,6-dichloro-benzeneacetamide (loviride), 1-[3-(isopropylamino)-2-pyridyl]-4-[5-(methanesulfonamido)-1H-indol-2-ylcarbonyl]piperazine monomethanesulfonate (delavirdine), (10R, 11S, 12S)-12-Hydroxy-6, 6, 10, 11-tetramethyl-4-propyl-11,12-dihydro-2H, 6H, 10H-benzo(1, 2-b:3, 4-b':5, 6-b")tripyran-2-one ((+) calanolide A), (4S)-6-Chloro-4-[1E)-cyclopropylethenyl)-3,4- dihydro-4-(trifluoromethyl)-2(1H)-quinazolinone (DPC-083), (S)-6-chloro-4-(cyclopropylethynyl)-1,4-dihydro-4-(trifluoromethyl)-2H-3,1-benzoxazin-2-one (efavirenz, DMP 266), 1-(ethoxymethyl)-5-(1-methylethyl)-6-(phenylmethyl)-2,4(1H,3H)-pyrimidinedione (MKC-442), and 5-(3,5-

dichlorophenyl)thio-4-isopropyl-1-(4-pyridyl)methyl-1H-imidazol-2-ylmethyl carbamate (capravirine), glycoprotein 120 antagonists, for example PRO-2000, PRO-542 and 1,4-bis[3-[(2, 4- dichlorophenyl)carbonylamino]-2-oxo-5,8-disodiumsulfanyl]naphthalyl-2, 5-dimethoxyphenyl-1, 4-dihydrazone (FP-21399), cytokine antagonists, for example reticulose (Product-R), 1,1'-azobis-formamide (ADA), 1,11-(1,4-phenylenebis(methylene))bis-1,4,8,11-tetraazacyclotetradecane octahydrochloride (AMD-3100), integrase inhibitors, or fusion inhibitors, for example T-20 and T-1249.

The present invention further includes the use of a compound according to the invention in the manufacture of a medicament for simultaneous or sequential administration with at least another therapeutic agent, such as those defined hereinbefore.

Compounds of the present invention may be administered with an agent known to inhibit or reduce the metabolism of compounds, for example ritonavir. Accordingly, the present invention features a method for the treatment including prophylaxis of a disease as hereinbefore described by administration of a compound of the present invention in combination with a metabolic inhibitor. Such combination may be administered simultaneously or sequentially.

In general a suitable dose for each of the above-mentioned conditions will be in the range of 0.01 to 250 mg per kilogram body weight of the recipient (e.g. a human) per day, suitably in the range of 0.1 to 100 mg per kilogram body weight per day and most suitably in the range 0.5 to 30 mg per kilogram body weight per day and particularly in the range 1.0 to 20 mg per kilogram body weight per day. Unless otherwise indicated, all weights of active ingredient are calculated as the parent compound of formula (I); for salts or esters thereof, the weights would be increased proportionally. The desired dose may be presented as one, two, three, four, five, six or more sub-doses administered at appropriate intervals throughout the day. In some cases the desired dose may be given on alternative days. These sub-doses may be administered in unit dosage forms, for example, containing 10 to 1000 mg or 50 to 500 mg, suitably 20 to 500 mg, and most suitably 50 to 400 mg of active ingredient per unit dosage form.

While it is possible for the active ingredient to be administered alone it is preferable to present it as a pharmaceutical composition. The compositions of the present invention comprise at least one active ingredient, as defined above, together with one or more acceptable carriers thereof and optionally other therapeutic agents. Each carrier must be acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the patient.

Pharmaceutical compositions include those suitable for oral, rectal, nasal, topical (including transdermal, buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, and intravitreal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods represent a further feature of the present invention and include the step of bringing into association the active ingredients with the carrier, which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

The present invention further includes a pharmaceutical composition as hereinbefore defined wherein a compound of the present invention or a pharmaceutically acceptable derivative thereof and another therapeutic agent are presented separately from one another as a kit of parts.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active compound 1) in an optionally buffered, aqueous solution or 2) dissolved and/or dispersed in an adhesive or 3) dispersed in a polymer. A suitable concentration of the active compound is about 1% to 25%, suitably about 3% to 15%. As one particular possibility, the active compound may be delivered from the patch by electrotransport or iontophoresis as generally described in Pharmaceutical Research 3 (6), 318 (1986).

Pharmaceutical compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, caplets, cachets or tablets each containing a predetermined amount of the active ingredients; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredients in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (e.g. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface-active or dispersing agent. Molded tablets may be made by molding a mixture of the powdered compound moistened with an inert liquid diluent in a suitable machine. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredients therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Pharmaceutical compositions suitable for topical administration in the mouth include lozenges comprising the active ingredients in a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray Pharmaceutical compositions containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Pharmaceutical compositions for rectal administration may be presented as a suppository with a suitable carrier comprising, for example, cocoa butter or a salicylate or other materials commonly used in the art. The suppositories may be

conveniently formed by admixture of the active combination with the softened or melted carrier(s) followed by chilling and shaping in molds.

Pharmaceutical compositions suitable for parenteral administration include aqueous and nonaqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the pharmaceutical composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents; and liposomes or other microparticulate systems which are designed to target the compound to blood components or one or more organs. The pharmaceutical compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injection, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Unit dosage pharmaceutical compositions include those containing a daily dose or daily subdose of the active ingredients, as hereinbefore recited, or an appropriate fraction thereof.

It should be understood that in addition to the ingredients particularly mentioned above the pharmaceutical compositions of this invention may include other agents conventional in the art having regard to the type of pharmaceutical composition in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavoring agents.

EXAMPLES

The following examples are intended for illustration only and are not intended to limit the scope of the invention in any way.

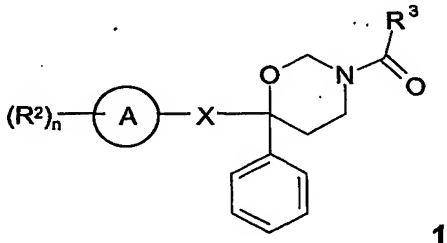
Low resolution, open-access LC-MS data were acquired in either ESI pos/neg or APCI pos/neg mode with scanning from 100-1100 amu @ 0.5 sec/scan.

LC conditions: flowrate 0.8 mL/min. 85% H2O (0.1% formic acid) to 100% MeOH (0.075% formic acid) in 6 minutes. Phenomenex Max-RP column, 2.0x50 mm.

High Resolution Mass Spectra were acquired using Micromass LCT mass spectrometer (time-of-flight) with flow injection (FIA-MS) at 0.3 mL/min with 100% MeOH (0.1% formic acid), run time of 2 minutes, in ESI+ mode, scanning from 100-1100 amu @ 0.5 sec/scan. Reserpine was used as the lock mass (m/z 609.2812) and to adjust mass scale.

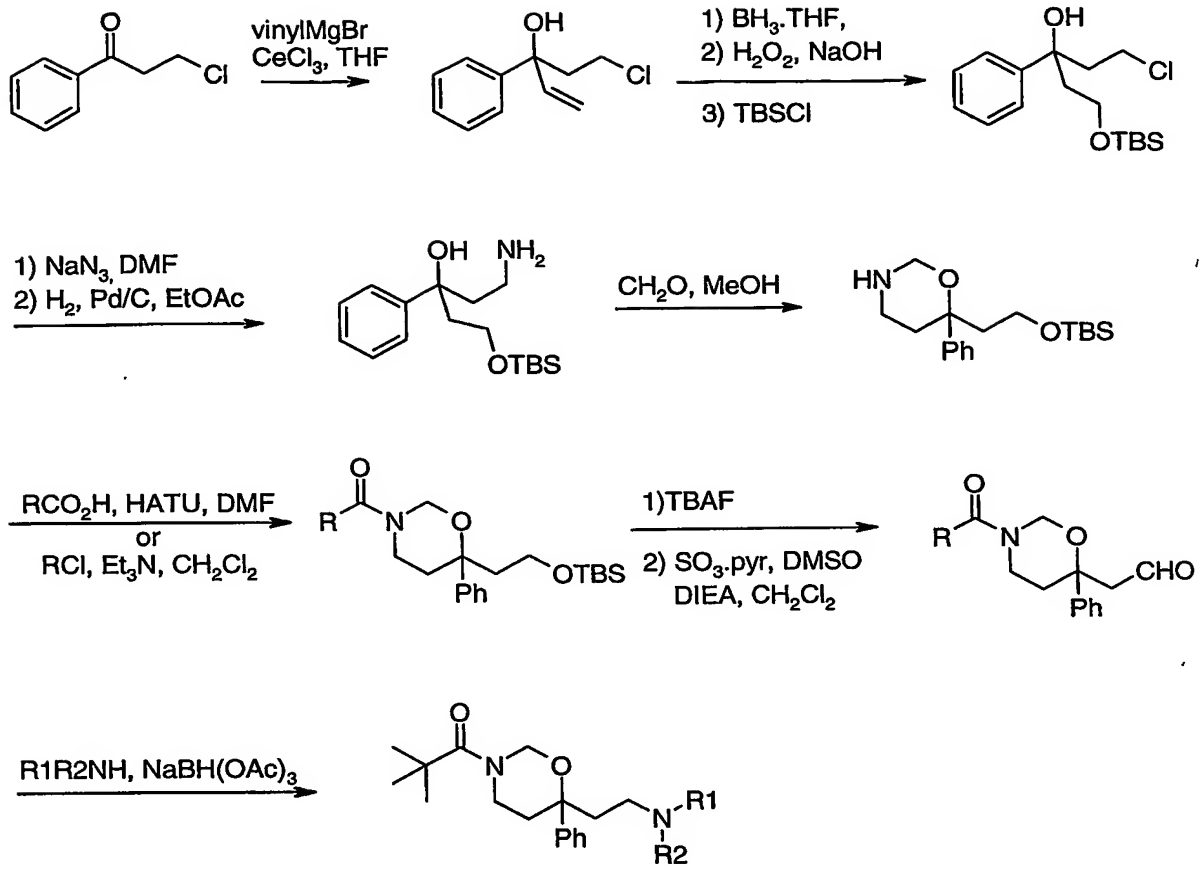
As will be appreciated by those skilled in the art, the following schemes may be followed in preparing the compounds of the present invention. Any variability depicted within the scheme(s) illustrated herein should be limited to the particular scheme and not necessarily extended throughout the rest of the present specification.

Scheme 1: Compounds of Formula **1**:

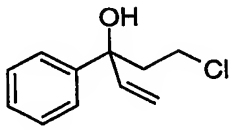


1

may be prepared according to the following general scheme:



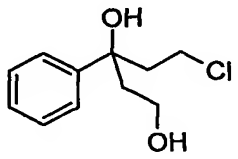
Preparation of 5-chloro-3-phenylpent-1-en-3-ol



Anhydrous $CeCl_3$ (16 g, 64.9 mmol) was stirred vigorously in 100 mL THF for 2h 15min. The suspension was then cooled to –78 °C and a 1M solution of vinylmagnesiumbromide (62 mL, 62.3 mmol) was added. After 1.5h at –78 °C, a solution of 3-chloropropiophenone (7g, 14.5 mmol) in 20 mL THF was added dropwise via cannula. The reaction mixture was stirred for 1h, then gradually warmed to room temperature and quenched by the addition of saturated aqueous $NaHCO_3$. Extraction into EtOAc (3X) followed by purification by silica gel chromatography (4:1 hexanes:EtOAc) afforded the 5-chloro-3-phenylpent-1-en-3-ol (7.7g, 94%) as a

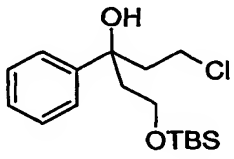
yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.34 (m, 4H), 7.28 (m, 1H), 6.18 (dd, 1H, J = 17.2, 10.6 Hz), 5.34 (d, 1H, J = 17.2 Hz), 5.23 (d, 1H, J = 10.8 Hz), 3.59 (td, 1H, J = 10.6, 5.5 Hz), 3.41 (td, 1H, J = 10.5, 5.5 Hz), 2.40 (m, 2H), 2.0 (br. s, 1H).

Preparation of 5-chloro-3-phenylpentane-1,3-diol



To a 0 °C solution of 5-chloro-3-phenylpent-1-en-3-ol (6.12g, 31.12 mmol) in 125 mL THF was added a 1M solution of BH_3.THF (62.2 mL, 62.2 mmol) dropwise. The reaction mixture was stirred for 3h at 0 °C, and 5M NaOH (38 mL, 190 mmol) was slowly added, followed by 30% H_2O_2 (56 mL, 495 mmol). After stirring for 90 min at room temperature, the organic layer was isolated and the aqueous layer extracted with EtOAc. The organic layers were pooled, washed with saturated aqueous $Na_2S_2O_3$ and saturated aqueous NH_4Cl, dried (Na_2SO_4), filtered, and passed through a silica plug to provide 5-chloro-3-phenylpentane-1,3-diol (4.3g, 64%) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43-7.34 (m, 5H), 7.27 (m, 1H), 3.80 (td, 1H, J = 10.8, 4.2 Hz), 3.67-3.52 (m, 2H), 3.22 (td, 1H, J = 10.5, 5.1 Hz), 2.38 (ddd, 1H, J = 13.7, 10.3, 5.9 Hz), 2.28 (ddd, 1H, J = 13.7, 10.4, 5.1 Hz), 2.23 (m, 1H), 2.03 (ddd, 1H, J = 14.8, 4.6, 2.9 Hz).

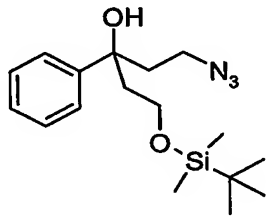
Preparation of 1-{[*tert*-butyl(dimethyl)silyl]oxy}-5-chloro-3-phenylpentan-3-ol



To a 0 °C solution of 5-chloro-3-phenylpentane-1,3-diol (4.3g, 20 mmol) and Et_3N (3.4 mL, 24 mmol) in 20 mL DMF, was added *tert*-butyldimethylsilyl chloride (3.3g, 21 mmol). After stirring for 2h at 0 °C, the reaction mixture was diluted with EtOAc, washed with saturated aqueous $NaHCO_3$, dried (Na_2SO_4) and chromatographed (9:1 hex:EtOAc) to provide 1-{[*tert*-butyl(dimethyl)silyl]oxy}-5-

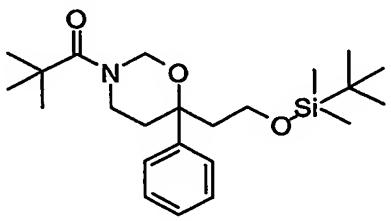
chloro-3-phenylpentan-3-ol (5.2g, 79%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.32 (m, 4H), 7.25 (m, 1H), 4.88 (s, 1H), 3.73 (dt, 1H, J = 10.3, 4.0 Hz), 3.65 (ddd, 1H, J = 11.5, 10.6, 5.1 Hz), 3.44 (td, 1H, J = 10.6, 2.7 Hz), 3.15 (ddd, 1H, J = 11.3, 10.6, 5.1 Hz), 2.34 (ddd, 1H, J = 13.3, 11.4, 5.1 Hz), 2.27-2.15 (m, 2H), 1.96 (dt, 1H, J = 14.6, 3.2 Hz), 0.86 (s, 9H), -0.02 (s, 3H), -0.09 (s, 3H); ESI-MS 351 (M+Na).

Preparation of 1-azido-5-{[*tert*-butyl(dimethyl)silyl]oxy}-3-phenylpentan-3-ol



A solution of 1-{[*tert*-butyl(dimethyl)silyl]oxy}-5-chloro-3-phenylpentan-3-ol ((5.2g, 15.7 mmol) and NaN_3 (7.8g , 120 mmol) in 50 mL DMF was stirred for 24h at 65 °C. The reaction mixture was then diluted with EtOAc, washed with water, dried, filtered and evaporated in vacuo to provide 1-azido-5-{[*tert*-butyl(dimethyl)silyl]oxy}-3-phenylpentan-3-ol in quantitative yield as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.32 (m, 4H), 7.24 (m, 1H), 4.89 (s, 1H), 3.72 (dt, 1H, J = 10.3, 3.9 Hz), 3.47-3.36 (m, 2H), 2.97 (ddd, 1H, J = 12.3, 10.1, 5.5 Hz), 2.24-2.10 (m, 2H), 2.07-1.93 (m, 2H), 0.86 (s, 9H), -0.02, (s, 3H), -0.09 (s, 3H); ESI-MS 358 (M+Na).

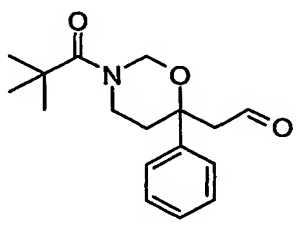
Preparation of 6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinane



A solution of 1-azido-5-{[*tert*-butyl(dimethyl)silyl]oxy}-3-phenylpentan-3-ol (1.1g, 3.3 mmol) in 30 mL EtOAc was stirred for 2h under 40 psi of H_2 gas in the presence of catalytic 10% Pd/C. After filtration through celite, the reaction mixture was evaporated to give 1-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-3-phenylpentan-3-ol in quantitative yield as a clear oil that was used without further purification. ESI-MS 310 (M+H), 332 (M+Na).

A solution of the above amino alcohol (288 mg, 0.93 mmol) and p-formaldehyde (300 mg, 10 mmol) in 9 mL MeOH was heated under reflux for 90 min. The reaction mixture was then filtered, concentrated in vacuo, and the residue was redissolved in 3 mL CH_2Cl_2, Et_3N (0.40 mL, 2.9 mmol) and trimethylacetyl chloride (0.23 mL, 1.9 mmol) were added, and the reaction was stirred for 24 h. After washing with saturated aqueous $NaHCO_3$, the organic phase was isolated, dried (Na_2SO_4), concentrated and chromatographed (3:1 hex:EtOAc) to provide 6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinane (290 mg, 71%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.41-7.34 (m, 4H), 7.28 (m, 1H), 5.41 (d, 1H, J = 10.6 Hz), 4.66 (d, 1H, J = 10.8 Hz), 4.21 (m, 1H), 3.55 (dt, 1H, J = 10.4, 7.2 Hz), 3.33 (dt, 1H, J = 10.4, 7.3 Hz), 3.06 (br. t, 1H, J = 11.8 Hz), 2.35 (ddd, 1H, J = 14.2, 4.3, 3.0 Hz), 2.07 (ddd, 1H, J = 14.3, 11.3, 4.2 Hz), 1.97 (m, 2H), 1.28 (s, 9H), 0.83 (s, 9H), -0.05 (s, 3H), -0.06 (s, 3H); ^{13}C NMR (100 MHz, $CHCl_3$) 176.9, 142.0, 128.7, 127.3, 126.4, 78.6, 72.0, 58.6, 46.4, 40.3, 38.8, 33.5, 28.5, 25.9, 18.2, -5.4; ESI-MS 406 (M+H), 428 (M+Na).

Preparation of [3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde



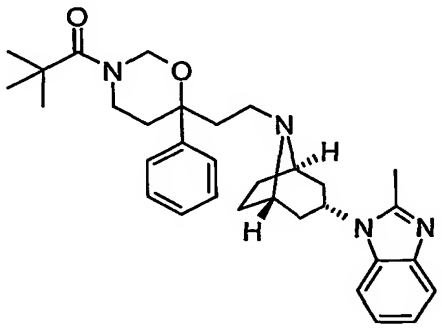
6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinane (4.07 g, 10.03 mmol) was stirred in a 1M solution of

tetrabutylammonium fluoride in THF (40 mL, 40 mmol) for 2h at room temperature. Saturated aqueous $NaHCO_3$ was added, and the reaction mixture was extracted with $CHCl_3$. The organic layers were dried (Na_2SO_4), concentrated and the residue was filtered through a silica plug flushing with 1:1 hex:EtOAc to afford 2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethanol in quantitative yield as a clear oil that was used without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 7.45-7.37 (m, 4H), 7.31 (m, 1H), 5.51 (dd, 1H, J = 10.9, 2.1 Hz), 4.69 (d, 1H, J = 11.0 Hz), 4.30 (m, 1H), 3.58 (m, 2H), 2.99 (m, 1H), 2.31 (dt, 1H, J = 14.1, 3.4 Hz), 2.16 (ddd, 1H, J = 14.1, 11.7, 4.4 Hz), 2.04 (ddd, 1H, J = 14.5, 7.0, 4.7 Hz), 1.92 (ddd, 1H, J = 14.6, 6.9, 4.7 Hz), 1.28 (s, 9H).

To a 0 °C solution of the above 2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethanol, dimethyl sulfoxide (10 mL, 141 mmol) and diisopropylethylamine (10.5 mL, 60 mmol) in 60 mL CH_2Cl_2 was added SO_3.pyr (9.0 g, 57 mmol) in one portion. The reaction mixture was stirred at 0 °C for 2h, quenched with saturated aqueous $NaHCO_3$, extracted with $CHCl_3$, dried (Na_2SO_4) and chromatographed (2:1 Hex:EtOAc) to provide [3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (2.84g, 98%) as an oil. ^1H NMR (400 MHz, $CDCl_3$): δ 9.64 (t, 1H, J = 2.7 Hz), 7.45-7.41 (m, 4H), 7.33 (m, 1H), 5.49 (dd, 1H, J = 11.1, 1.9 Hz), 4.74 (d, 1H, J = 11.5 Hz), 4.27 (m, 1H), 3.08 (m, 1H), 2.72 (AB_qd, 2H, J = 15.3, 2.8 Hz), 2.43 (ddd, 1H, J = 14.2, 4.1, 3.0 Hz), 2.11 (ddd, 1H, J = 14.2, 11.4, 4.3 Hz), 1.28 (s, 9H). ESI-MS 312 (M+Na).

Example 1

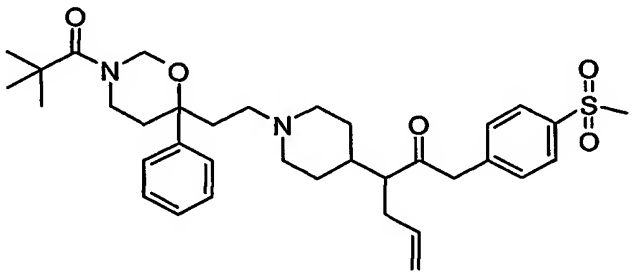
Preparation of 1-((1*R*,5*S*)-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole



To a solution of [3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (50.4 mg, 0.17 mmol), 1-[(1*R*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl]-2-methyl-1*H*-benzimidazole dihydrochloride (64 mg, 0.17 mmol) and diisopropylethylamine (0.063 mL, 0.35 mmol) in 2 mL CH_2Cl_2 was added sodium triacetoxyborohydride (55 mg, 0.26 mmol) and the reaction mixture was stirred for 2h at room temperature. After evaporation, the residue was chromatographed (5% (2M NH_3 / MeOH) in $CHCl_3$) to provide 1-((1*R*,5*S*)-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole (50.4 mg, 56%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (m, 1H), 7.43-7.34 (m, 4H), 7.32-7.26 (m, 2H), 7.19-7.11 (m, 2H), 5.43 (s, 1H, J = 11.2 Hz), 4.70 (d, 1H, J = 10.3 Hz), 4.61 (m, 1H), 4.21 (m, 1H), 3.42-3.22 (m, 2H), 3.08 (m, 1H), 2.57 (s, 3H), 2.43-1.57 (m, 14H), 1.28 (s, 9H); ESI-MS 515 (M+H), 237 (M+Na).

Example 2

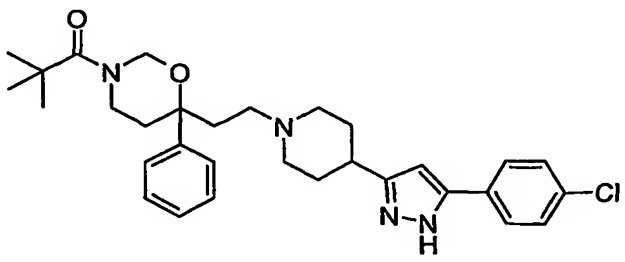
Preparation of 3-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1-[4-(methylsulfonyl)phenyl]hex-5-en-2-one



3-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1-[4-(methylsulfonyl)phenyl]hex-5-en-2-one (26 mg, 49%) was obtained as a white solid from [3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (25 mg, 0.087 mmol), 1-[4-(methylsulfonyl)phenyl]-3-piperidin-4-ylhex-5-en-2-one (44 mg, 0.13 mmol), diisopropylethylamine (0.033 mL, 0.19 mmol) and sodium triacetoxyborohydride (27 mg, 0.13 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.90-7.86 (m, 2H), 7.44-7.36 (m, 4H), 7.35-7.29 (m, 3H), 5.86 (m, 1H), 5.44 (d, 1H, J = 10.4 Hz), 5.31-5.18 (m, 2H), 4.83-4.59 (m, 2H), 4.15-3.83 (m, 3H), 3.73 (s, 2H), 3.29-1.66 (m, 15H), 3.04 (s, 3H), 1.26 (s, 9H); ESI-MS 610 (M+H), 632 (M+Na).

Example 3

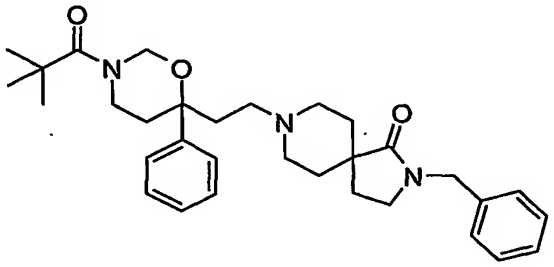
Preparation of 6-(2-{4-[5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]piperidin-1-yl}ethyl)-3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinane



6-(2-{4-[5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]piperidin-1-yl}ethyl)-3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinane (35.0 mg, 75%) was obtained as a white solid from [3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (25 mg, 0.087 mmol), 4-[5-(4-chlorophenyl)-1*H*-pyrazol-3-yl]piperidine (34 mg, 0.13 mmol), diisopropylethylamine (0.033 mL, 0.19 mmol) and sodium triacetoxyborohydride (27 mg, 0.13 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.77-7.50 (m, 2H), 7.45-7.28 (m, 7H), 6.33 (s, 1H), 5.44 (d, 1H, J = 11.0 Hz), 4.79 (m, 1H), 4.12 (m, 1H), 3.37-2.98 (m, 3H), 2.77 (m, 1H), 2.51-1.42 (m, 13H), 1.28 (s, 9H); ESI-MS 535 (M+H), 557 (M+Na).

Example 4

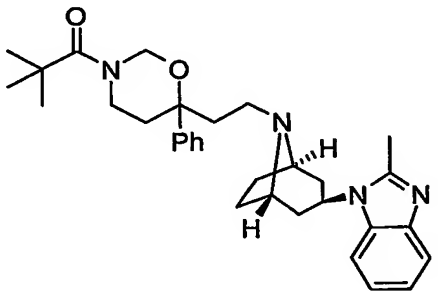
Preparation of 2-benzyl-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-2,8-diazaspiro[4.5]decan-1-one



2-benzyl-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-2,8-diazaspiro[4.5]decan-1-one (25mg, 56%) was obtained as a white solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (25 mg, 0.087 mmol), 2-benzyl-2,8-diazaspiro[4.5]decan-1-one (37 mg, 0.13 mmol), diisopropylethylamine (0.033 mL, 0.19 mmol) and sodium triacetoxyborohydride (27 mg, 0.13 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.42-7.27 (m, 8H), 7.19-7.15 (m, 2H), 5.42 (d, 1H, J = 11.2 Hz), 4.76 (m, 1H), 4.40 (s, 2H), 4.13 (m, 1H), 3.52 (m, 1H), 3.25-3.00 (m, 3H), 2.74 (m, 1H), 2.37-1.51 (m, 13H), 1.28 (s, 9H); ESI-MS 518 (M+H).

Example 5

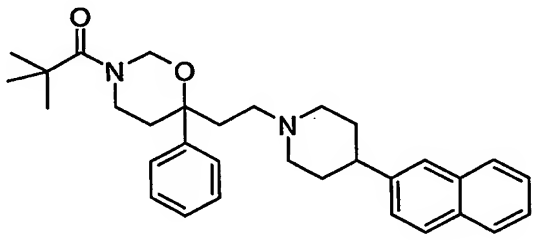
Preparation of 1-((1*R*,5*S*)-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole



1-((1*R*,5*S*)-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole (22.8 mg, 44%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 1-[(1*R*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl]-2-methyl-1*H*-benzimidazole (24.1 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.70 (m, 1H), 7.59-7.44 (m, 5H), 7.37 (m, 1H), 7.28-7.18 (m, 2H), 5.49 (d, 1H, J = 10.8 Hz), 4.82 (d, 1H, J = 10.8 Hz), 4.53 (m, 1H), 4.27 (m, 1H), 3.51-3.33 (m, 2H), 3.20 (m, 1H), 2.61 (s, 3H), 2.70-2.29 (m, 5H), 2.26-1.94 (m, 5H), 1.80-1.54 (m, 4H), 1.34 (s, 9H); ESI-MS 515 (M+H).

Example 6

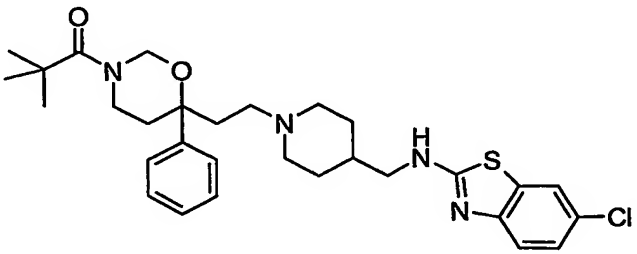
Preparation of 3-(2,2-dimethylpropanoyl)-6-{2-[4-(2-naphthyl)piperidin-1-yl]ethyl}-6-phenyl-1,3-oxazinane



3-(2,2-dimethylpropanoyl)-6-{2-[4-(2-naphthyl)piperidin-1-yl]ethyl}-6-phenyl-1,3-oxazinane (28.0 mg, 58%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 4-(2-naphthyl)piperidine (24.8 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.93-7.76 (m, 3H), 7.70-7.32 (m, 9H), 5.47 (d, 1H, J = 10.9 Hz), 4.81 (m, 1H), 4.24 (br., 1H), 3.27-2.80 (m, 5H), 2.75-1.84 (m, 11H), 1.34 (s, 9H); ESI-MS 485 (M+H).

Example 7

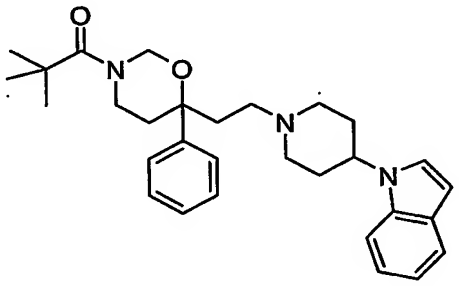
Preparation of 6-chloro-*N*-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]-1,3-benzothiazol-2-amine



6-chloro-*N*-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]-1,3-benzothiazol-2-amine (25.4 mg, 46%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 6-chloro-*N*-(piperidin-4-ylmethyl)-1,3-benzothiazol-2-amine (17.5 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.66-7.55 (m, 1H), 7.50-7.26 (m, 7H), 5.65 (br., 1H), 5.45 (d, 1H, J = 10.9 Hz), 4.77 (d, 1H, J = 10.8 Hz), 4.21 (m, 1H), 3.47-3.29 (m, 2H), 3.25-2.91 (m, 3H), 2.55-1.30 (m, 13H), 1.32 (s, 9H); ESI-MS 555 (M+H).

Example 8

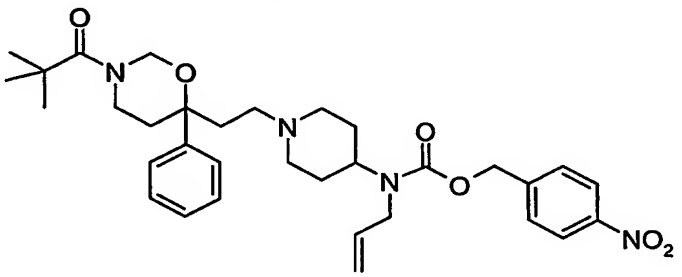
Preparation of 1-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1*H*-indole



1-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1*H*-indole (28.1 mg, 59%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 1-piperidin-4-yl-1*H*-indole (23.7 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.61 (d, 1H, J = 7.7 Hz), 7.45-7.35 (m, 4H), 7.36-7.29 (m, 2H), 7.08 (t, 1, J = 7.2 Hz), 6.50 (d, 1H, J = 3.2 Hz), 5.43 (d, 1H, J = 10.8 Hz), 4.72 (d, 1H, J = 10.8 Hz), 4.29-4.14 (m, 2H), 3.16-2.95 (m, 3H), 2.45-1.90 (m, 12H), 1.29 (s, 9H); ESI-MS 474 (M+H), 486 (M+Na).

Example 9

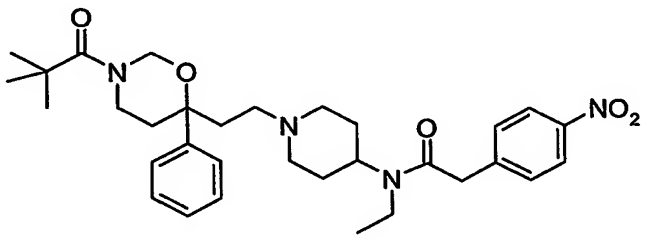
Preparation of 4-nitrobenzyl allyl(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)carbamate



4-nitrobenzyl allyl(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)carbamate (34.1 mg, 57%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 4-nitrobenzyl allyl(piperidin-4-yl)carbamate (31.9 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.19 (m, 2H), 7.46 (m, 2H), 7.40-7.31 (m, 4H), 7.28 (m, 1H), 5.76 (m, 1H), 5.39 (d, 1H, J = 11.0 Hz), 5.23-5.03 (m, 4H), 4.67 (d, 1H, J = 10.8 Hz), 4.20 (m, 1H), 3.98 (br., 1H), 3.87-3.74 (m, 3H), 3.10-2.77 (m, 3H), 2.36-1.57 (m, 11H), 1.26 (s, 9H); ESI-MS 593 (M+H), 615 (M+Na).

Example 10

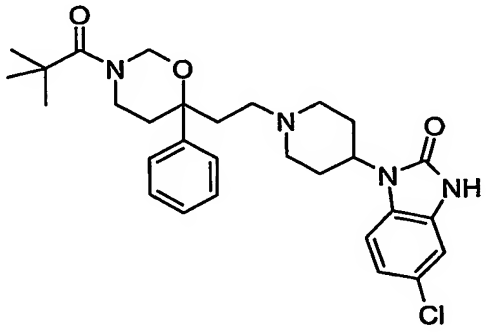
Preparation of *N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-*N*-ethyl-2-(4-nitrophenyl)acetamide



N-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-*N*-ethyl-2-(4-nitrophenyl)acetamide (36.6 mg, 65%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-ethyl-2-(4-nitrophenyl)-*N*-piperidin-4-ylacetamide (29.1 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.15(m, 2H), 7.43-7.26 (m, 7H), 5.39 (d, 1H, J = 10.9 Hz), 4.66 (m, 1H), 4.36 (m, 1H), 4.19 (m, 1H), 3.76 (m, 2H), 3.27 (m, 3H), 3.09-2.74 (m, 4H), 2.36-1.53 (m, 10H), 1.26 (s, 9H), 1.09 (m, 3H); ESI-MS 565 (M+H), 587 (M+Na).

Example 11

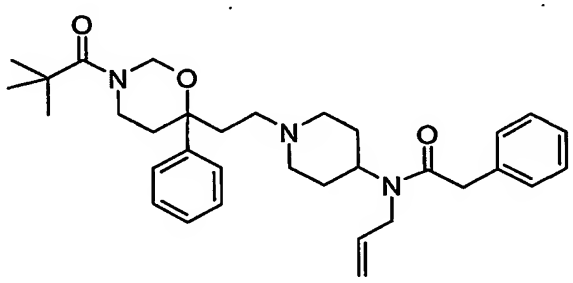
Preparation of 5-chloro-1-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one



5-chloro-1-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-1,3-dihydro-2*H*-benzimidazol-2-one (32.9 mg, 62%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 5-chloro-1-piperidin-4-yl-1,3-dihydro-2*H*-benzimidazol-2-one (25.2 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 9.70 (s, 1H), 7.44-7.27 (m, 5H), 7.22-7.05 (m, 2H), 7.01 (m, 1H), 5.44 (d, 1H, J = 10.8 Hz), 4.71 (d, 1H, J = 11.1 Hz), 4.35-4.16 (m, 2H), 3.16-2.93 (m, 3H), 2.50-1.88 (m, 11H), 1.83-1.67 (m, 2H), 1.29 (s, 9H); ESI-MS 525 (M+H).

Example 12

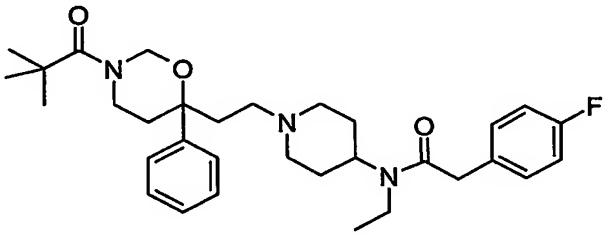
Preparation of *N*-allyl-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-2-phenylacetamide



N-allyl-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-2-phenylacetamide (34.0 g, 64%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-allyl-2-phenyl-*N*-piperidin-4-ylacetamide (25.8 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.40-7.16 (m, 10H), 5.78 (m, 1H), 5.39 (d, 1H, J = 11.1 Hz), 5.24-5.13 (m, 2H), 5.04 (m, 1H), 4.67 (m, 1H), 4.49 (m, 1H), 4.19 (m, 1H), 3.94-3.46 (m, 5H), 3.14-2.65 (m, 3H), 2.36-1.49 (m, 10H), 1.26 (s, 9H); ESI-MS 532 (M+H).

Example 13

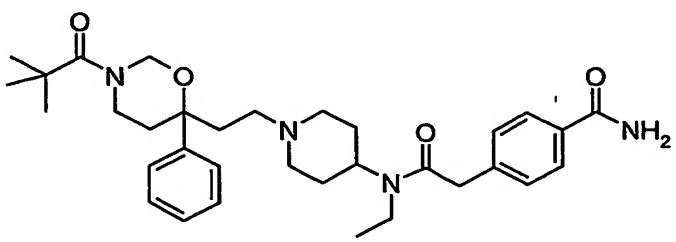
Preparation of *N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-*N*-ethyl-2-(4-fluorophenyl)acetamide



N-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)-*N*-ethyl-2-(4-fluorophenyl)acetamide (34.0 mg, 63%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-ethyl-2-(4-fluorophenyl)-*N*-piperidin-4-ylacetamide (26.4 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.41-7.26 (m, 5H), 7.21-7.14 (m, 2H), 7.00-6.94 (m, 2H), 5.39 (d, 1H, J = 10.8 Hz), 4.67 (m, 1H), 4.37 (m, 1H), 4.19 (m, 1H), 3.69-3.59 (m, 2H), 3.51-2.67 (m, 8H), 2.36-1.54 (m, 9H), 1.26 (s, 9H), 1.15 (m, 3H); ESI-MS 538 (M+H), 560 (M+Na).

Example 14

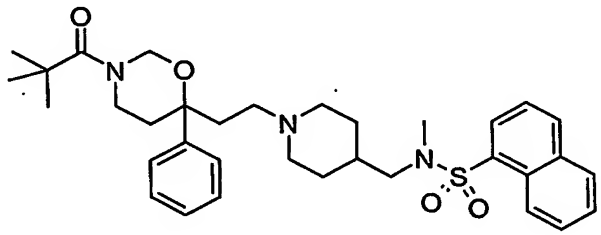
Preparation of 4-{2-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)(ethyl)amino]-2-oxoethyl}benzamide



4-{2-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)(ethyl)amino]-2-oxoethyl}benzamide (14.5 mg, 26%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), 4-{2-[ethyl(piperidin-4-yl)amino]-2-oxoethyl}benzamide (28.9 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.75 (d, 2H, J = 8.1 Hz), 7.41-7.35 (m, 2H0, 7.35-7.28 (m, 5H), 6.11 (br., 1H), 5.61 (br., 1H), 5.41 (m, 1H), 4.70 (m, 1H), 4.47 (br., 1H), 4.18 (m, 1H), 3.73 (m, 2H), 3.33-3.24 (m, 2H), 3.16-2.99 (m, 2H), 2.78 (br., 1H), 2.34-1.50 (m, 10H), 1.27 (s, 9H), 1.15 (m, 3H); ESI-MS 563 (M+H), 585 (M+Na).

Example 15

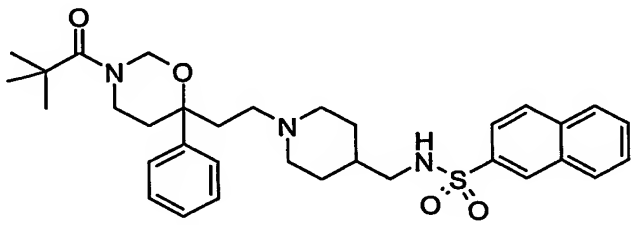
Preparation of *N*-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]-*N*-methylnaphthalene-1-sulfonamide



N-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]-*N*-methylnaphthalene-1-sulfonamide (44.2 mg, 75%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-methyl-*N*-(piperidin-4-ylmethyl)naphthalene-1-sulfonamide (31.8 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.71 (d, 1H, J = 8.1 Hz), 8.16 (d, 1H, J = 7.3 Hz), 8.09 (d, 1H, J = 8.0 Hz), 7.95 (d, 1H, J = 7.9 Hz), 5.42 (d, 1H, J = 10.8 Hz), 4.77 (d, 1H, J = 10.8 Hz), 4.46-4.09 (m, 3H), 3.25-0.85 (m, 17H), 1.34 (s, 9H); ESI-MS 592 (M+H).

Example 16

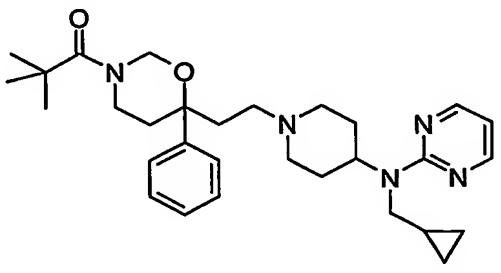
Preparation of *N*-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]naphthalene-2-sulfonamide



N-[(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)methyl]naphthalene-2-sulfonamide (26.4 mg, 46%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-(piperidin-4-ylmethyl)naphthalene-2-sulfonamide (30.4 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.40 (s, 1H), 7.98-7.87 (m, 3H), 7.81 (dd, 1H, J = 8.7, 1.7 Hz), 7.67-7.57 (m, 2H), 7.40-7.25 (m, 5H), 5.38 (d, 1H, J = 10.9 Hz), 5.15 (br., 1H), 4.75 (d, 1H, J = 11.0 Hz), 4.08 (m, 1H), 3.21-2.92 (m, 3H), 2.81 (br., 2H), 2.48 (m, 1H), 2.33-2.11 (m, 3H), 2.09-1.90 (m, 4H), 1.75-1.62 (m, 2H), 1.61-1.35 (m, 3H), 1.25 (s, 9H); ESI-MS 578 (M+H).

Example 17

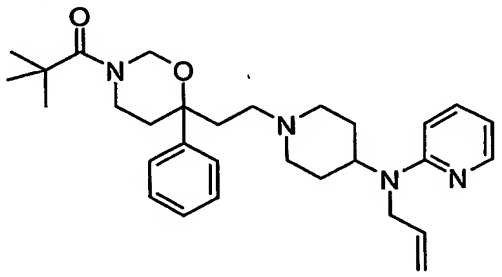
Preparation of *N*-(cyclopropylmethyl)-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)pyrimidin-2-amine



N-(cyclopropylmethyl)-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)pyrimidin-2-amine (29.1 mg, 58%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-(cyclopropylmethyl)-*N*-piperidin-4-ylpyrimidin-2-amine (26.9 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.26 (d, 2H, J = 4.7 Hz), 7.42-7.27 (m, 5H), 6.44 (t, 1H, J = 4.7 Hz), 5.41 (d, 1H, J = 10.8 Hz), 4.77 (d, 1H, J = 11.0 Hz), 4.57 (m, 1H), 4.12 (m, 1H), 3.37 (d, 2H, J = 6.5 Hz), 3.23-3.08 (m, 3H), 2.58 (br., 1H), 2.42-2.20 (m, 5H), 2.15-2.00 (m, 4H), 1.83-1.70 (m, 2H), 1.27 (s, 9H), 1.10 (m, 1H), 0.45 (m, 2H), 0.32 (m, 2H); ESI-MS 506 (M+H), 528 (M+Na).

Example 18

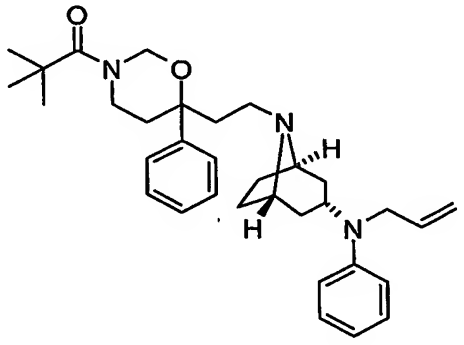
Preparation of *N*-allyl-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)pyridin-2-amine



N-allyl-*N*-(1-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}piperidin-4-yl)pyridin-2-amine (36.0 mg, 73%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), *N*-allyl-*N*-piperidin-4-ylpyridin-2-amine (25.4 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.07 (m, 1H), 7.42-7.27 (m, 6H), 6.51 (dd, 1H, J = 6.8, 5.2 Hz), 6.39 (d, 1H, J = 8.5 Hz), 5.80 (m, 1H), 5.41 (d, 1H, J = 11.0 Hz), 5.16-5.06 (m, 2H), 4.76 (d, 1H, J = 10.9 Hz), 4.12 (m, 1H), 3.89 (s, 2H), 3.20-3.02 (m, 3H), 2.55 (br., 1H), 2.38-166 (m, 12H), 1.27 (s, 9H); ESI-MS 491 (M+H), 513 (M+Na).

Example 19

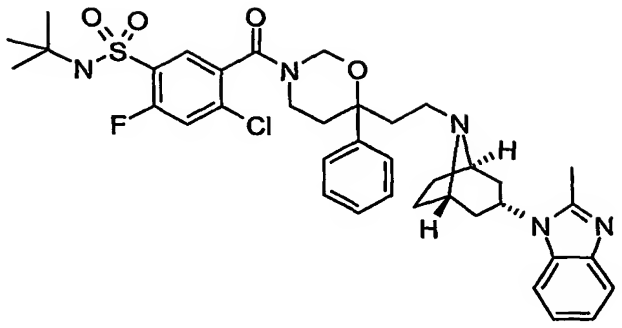
Preparation of (1*R*,5*S*)-*N*-allyl-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-*N*-phenyl-8-azabicyclo[3.2.1]octan-3-amine



(1*R*,5*S*)-*N*-allyl-8-{2-[3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]ethyl}-*N*-phenyl-8-azabicyclo[3.2.1]octan-3-amine (31.2 mg, 60%) was obtained as a solid from 3-(2,2-dimethylpropanoyl)-6-phenyl-1,3-oxazinan-6-yl]acetaldehyde (30 mg, 0.10 mmol), (1*R*,5*S*)-*N*-allyl-*N*-phenyl-8-azabicyclo[3.2.1]octan-3-amine (27.9 mg, 0.1 mmol), and polymer supported - triacetoxyborohydride (200 mg, 0.6 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.43-7.25 (m, 7H), 7.03 (m, 1H), 6.96 (m, 1H), 5.68 (m, 1H), 5.41 (d, 1H, J = 10.7 Hz), 5.05 (d, 1H, J = 10.3 Hz), 4.98 (d, 1H, J = 16.9 Hz), 4.89 (d, 1H, J = 10.9 Hz), 3.96 (m, 1H), 3.78 (m, 1H), 3.71 (d, 2H, J = 6.6 Hz), 3.62-3.30 (m, 4H), 2.88-1.60 (m, 13H), 1.26 (m, 6H); ESI-MS 516 (M+H), 538 (M+Na).

Example 20

Preparation of *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-[(6-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-6-phenyl-1,3-oxazinan-3-yl)carbonyl]benzenesulfonamide



Preparation of *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-{[6-(2-hydroxyethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}benzenesulfonamide. A solution of 1-amino-5-{[*tert*-butyl(dimethyl)silyl]oxy}-3-phenylpentan-3-ol (278 mg, 0.9 mmol) and p-formaldehyde (300 mg, 10 mmol) in 9 mL MeOH was heated under reflux for 90 min. A 2 mL aliquot (0.2 mmol) was removed, filtered, and concentrated in vacuo. The residue was redissolved in 0.5 mL DMF and 1 mL CH_2Cl_2 and to this solution was added 5-[(*tert*-butylamino)sulfonyl]-2-chloro-4-fluorobenzoic acid (62 mg, 0.2 mmol), diisopropylethylamine (70 μL, 0.4 mmol) and HATU (114 mg, 0.3 mmol). After 5h, saturated aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with $CHCl_3$. The organic layers were pooled, dried (Na_2SO_4), concentrated and purified by chromatography (4:1 hex:EtOAc) to provide *N*-(*tert*-butyl)-5-{[6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}-4-chloro-2-fluorobenzenesulfonamide, which was used without further purification.

The crude *N*-(*tert*-butyl)-5-{[6-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}-4-chloro-2-fluorobenzenesulfonamide was stirred in a solution of 1M TBAF in THF for 1.5h, then washed with saturated aqueous $NaHCO_3$ and purified by chromatography (2:1 EtOAc:hex) to provide *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-{[6-(2-hydroxyethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}benzenesulfonamide (25.2 mg, 25%) as a clear oil. ^1H NMR (400 MHz, $CDCl_3$), δ 8.03 (d, 0.5 H, J = 7.3 Hz, rotamer), 7.70 (d, 0.5 H, J = 7.3 Hz, rotamer), 7.46-7.30 (m, 6H), 5.81 (d, 0.5 H, J = 10.4 Hz, rotamer), 4.98 (m, 0.5 H, rotamer), 4.88 (m, 1H), 4.72-4.48 (m, 3H), 3.15 (m, 1H), 2.96 (m, 1H), 2.52-1.73 (m, 5H), 1.24 (m, 9H, rotamers); ESI-MS 497 (M-H).

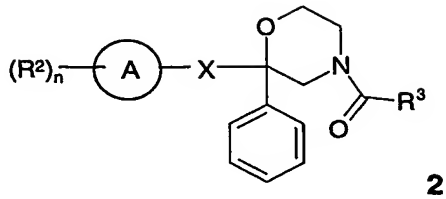
To a 0 °C solution of *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-{[6-(2-hydroxyethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}benzenesulfonamide (25.2 mg, 0.05 mmol) in dimethyl sulfoxide (71 μL, 1 mmol), diisopropylethylamine (53μL, 0.30 mmol) and 1mL CH_2Cl_2

was added SO_3.pyr in one portion. The reaction mixture was stirred for 2h at 0 °C, saturated aqueous $NaHCO_3$ was added, and the aqueous layer was extracted with $CHCl_3$. After evaporation, the resultant crude *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-{[6-(2-oxoethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}benzenesulfonamide was obtained and used in the next step without further purification.

N-(*tert*-butyl)-4-chloro-2-fluoro-5-[(6-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-6-phenyl-1,3-oxazinan-3-yl)carbonyl]benzenesulfonamide (25.3 mg, 70%) was obtained as a white solid from the above crude *N*-(*tert*-butyl)-4-chloro-2-fluoro-5-{[6-(2-oxoethyl)-6-phenyl-1,3-oxazinan-3-yl]carbonyl}benzenesulfonamide, 1-[(1*R*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl]-2-methyl-1*H*-benzimidazole dihydrochloride (18.6 mg, 0.05 mmol), diisopropylethylamine (18 μL, 0.10 mmol) and sodium triacetoxyborohydride (16 mg, 0.08 mmol) following the procedure in example 1. ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.03 (d, 0.5H, J = 7.1 Hz, rotamer), 7.72 (d, 0.5H, J = 7.3 Hz, rotamer), 7.65 (m, 1H), 7.45-7.39 (m, 2H), 7.37-7.28 (m, 5H), 7.20-7.11 (m, 2H), 5.73 (d, 0.5H, J = 10.4 ZHz, rotamer), 5.04-4.96 (m, 1H), 4.75-4.44 (m, 3.5H, rotamers), 3.42-3.13 (m, 3H), 2.63-2.54 (m, 5H), 2.52-1.86 (m, 10H), 1.74-1.63 (br., 2H), 1.29-1.20 (m, 9H, rotamers); ESI-MS 722 (M+H).

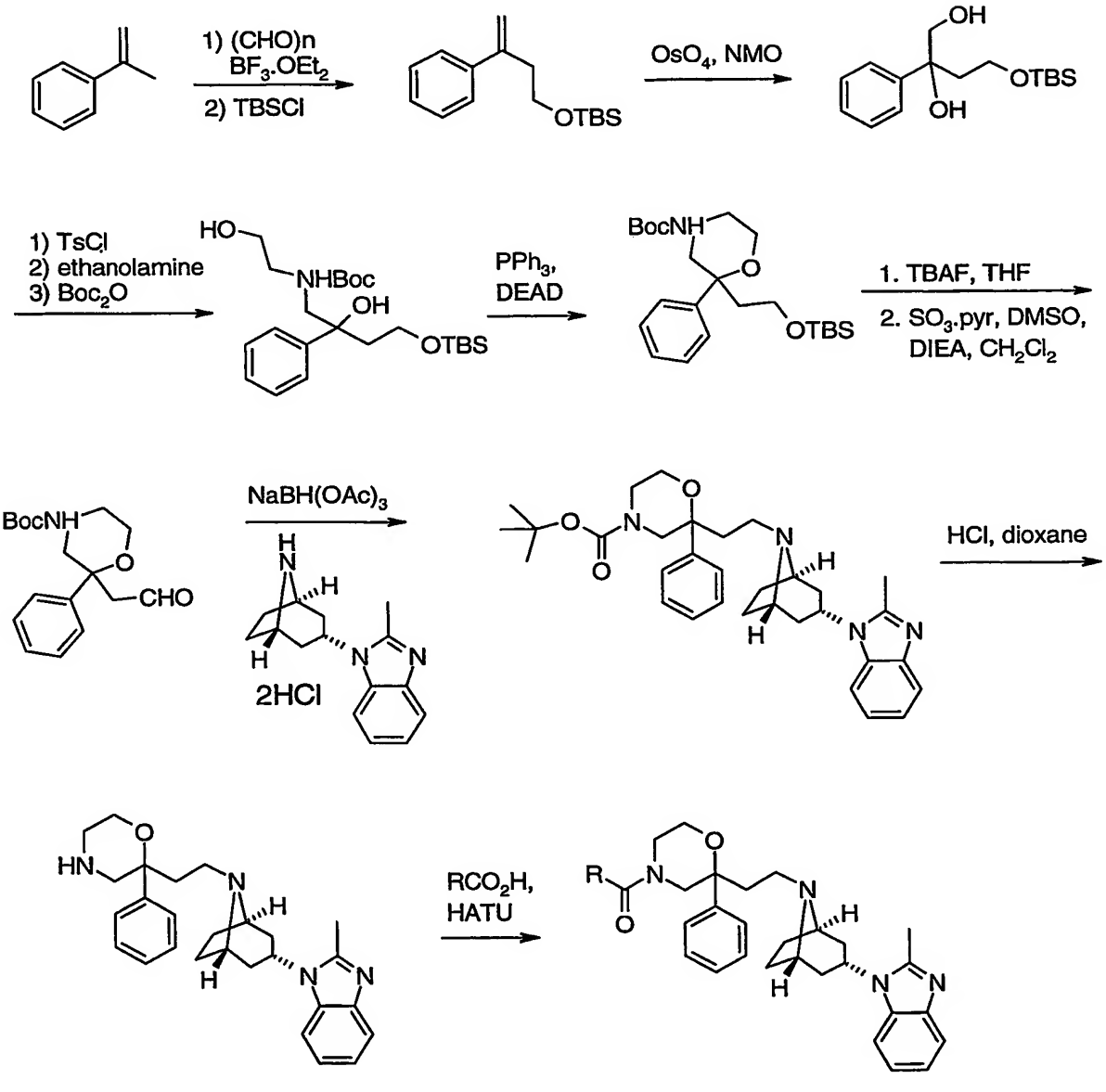
Those skilled in the art will appreciate such an example as a teaching of the scope of the present invention.

Scheme 2: Compounds of Formula **2**:

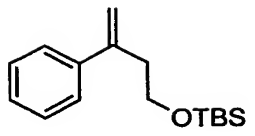


2

may be prepared according to the following general scheme:



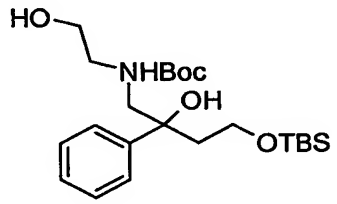
Preparation of *tert*-butyl(dimethyl)[(3-phenylbut-3-enyl)oxy]silane



3-phenylbut-3-en-1-ol was prepared according to the literature reference: Omaka et al. *Org. Lett.* **2002**, *4*, 1667.

To a 0 °C solution of 3-phenylbut-3-en-1-ol (1.0 g, 6.7 mmol) and Et_3N (0.59 mL, 8.1 mmol) in 7 mL DMF was added TBSCl (1.2 g, 8.1 mmol). After stirring for 4h at 0 °C, the reaction mixture was diluted with 4:1 EtOAc:hex and washed with water and chromatographed (5% EtOAc in hex) to provide *tert*-butyl(dimethyl)[(3-phenylbut-3-enyl)oxy]silane (794 mg, 45%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.43-7.40 (m, 2H), 7.35-7.30 (m, 2H), 7.26 (m, 1H), 5.34 (d, 1H, J = 1.5 Hz), 5.10 (m, 1H), 3.71 (t, 2H, J = 7.1 Hz), 2.74 (td, 2H, J = 7.1, 1.0 Hz), 0.87 (s, 9H), 0.00 (s, 6H); ESI-MS 263 (M+H).

Preparation of *tert*-butyl 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-hydroxy-2-phenylbutyl(2-hydroxyethyl)carbamate

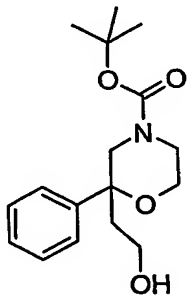


To a solution of *tert*-butyl(dimethyl)[(3-phenylbut-3-enyl)oxy]silane (526 mg, 2.0 mmol) and 4-methylmorpholine N-oxide (1.15 mL of a 60% solution in water, 5.9 mmol) in 3 mL acetone and 0.75 mL pH 7 buffer was added OsO_4 (0.17 mL of a 4% solution in water, 0.03 mmol) and the reaction mixture was stirred for 24h. Saturated aqueous $Na_2S_2O_3$ was added and stirred for 30 min and the mixture was then diluted with EtOAc, and washed with saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4), and passed through a short silica plug to afford 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-phenylbutane-1,2-diol as a clear oil that was used without further purification: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.45-7.41 (m, 2H), 7.39-7.34 (m, 2H), 7.27 (m, 1H), 4.88

(s, 1H), 3.77 (ddd, 1H, J = 10.3, 4.1, 3.6 Hz), 3.67 (ddd, 1H, J = 11.1, 8.4, 1.2 Hz), 3.62-3.54 (m, 2H), 2.51 (dd, 1H, J = 8.6, 4.8 Hz), 2.41 (ddd, 1H, J = 14.8, 11.3, 4.2 Hz), 1.91 (ddd, 1H, J = 14.8, 3.3, 2.7 Hz), 1.55 (s, 1H), 0.88 (s, 9H), 0.01 (s, 3H), -0.04 (s, 3H).

To a solution of the above 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-phenylbutane-1,2-diol in 2 mL pyridine was added p-toluenesulfonyl chloride (573 mg, 3.00 mmol). After stirring the reaction mixture for 24h, water was added and the solution was extracted with EtOAc. The organic layers were pooled and washed with brine, dried (Na_2SO_4), evaporated and redissolved in 5 mL CH_3CN. To this solution was added ethanolamine (0.61 mL, 10.0 mmol) and the reaction mixture was heated at 100 °C for 24h. After dilution with EtOAc, the mixture was washed with brine, dried (Na_2SO_4), evaporated and redissolved in 2 mL CH_2Cl_2. To this was added Et_3N (0.40 mL, 2.9 mmol), and di-*tert*-butyl dicarbonate (570 mg, 2.6 mmol) and the reaction mixture was stirred for 24 h. Water was added and the solution as extracted with CH_2Cl_2 and purified by chromatography (3:1 hex:EtOAc) to afford *tert*-butyl 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-hydroxy-2-phenylbutyl(2-hydroxyethyl)carbamate (773 mg, 88%) as a clear oil: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.48 (m, 1H), 7.40 (m, 1H), 7.37-7.31 (m, 2H), 7.25 (m, 1H), 3.99 (d, 1H, J = 14.1 Hz), 3.88-3.59 (m, 4H), 3.50-3.41 (m, 2H), 3.27 (m, 1H), 3.14 (m, 1H), 2.34-2.13 (m, 2H), 2.06 (m, 1H), 1.45 (s, 9H), 0.83 (s, 9H), -0.05 (s, 3H), -0.13 (s, 3H).

Preparation of *tert*-butyl 2-(2-hydroxyethyl)-2-phenylmorpholine-4-carboxylate

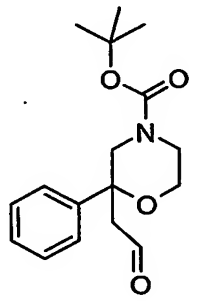


To a solution of *tert*-butyl 4-{[*tert*-butyl(dimethyl)silyl]oxy}-2-hydroxy-2-phenylbutyl(2-hydroxyethyl)carbamate (770 mg, 1.7 mmol) and triphenylphosphine (551 mg, 2.1 mmol) in 8 mL toluene was added diethyl azodicarboxylate (0.96 mL of a 40% solution in toluene, 2.1 mmol) and the reaction was stirred for 24 h. After

evaporation, the residue was chromatographed (10% EtOAc/hex) to afford *tert*-butyl 2-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-2-phenylmorpholine-4-carboxylate as an oil, which was used without further purification. ESI-MS 422 (M+H).

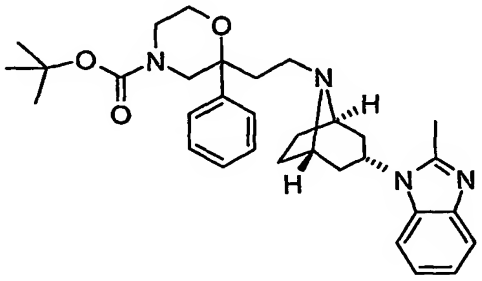
The above *tert*-butyl 2-(2-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-2-phenylmorpholine-4-carboxylate was stirred in 4 mL of a 1M solution of tetrabutylammonium fluoride in THF for 1 h at room temperature. The reaction mixture was then diluted with $CHCl_3$, washed with saturated aqueous $NaHCO_3$, and purified (1:1 hex:EtOAc) to provide *tert*-butyl 2-(2-hydroxyethyl)-2-phenylmorpholine-4-carboxylate (449 mg, 83%) as an oil: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.47-7.41 (m, 2H), 7.40-7.35 (m, 2H), 7.28 (m, 1H), 4.34 (m, 1H), 3.77-3.63 (m, 2H), 3.63-3.48 (m, 3H), 3.35 (m, 1H), 3.17 (m, 1H), 2.08 (m, 1H), 1.89 (m, 1H), 1.50-1.37 (br., 9H); ESI-MS 330 (M+Na).

Preparation of *tert*-butyl 2-(2-oxoethyl)-2-phenylmorpholine-4-carboxylate



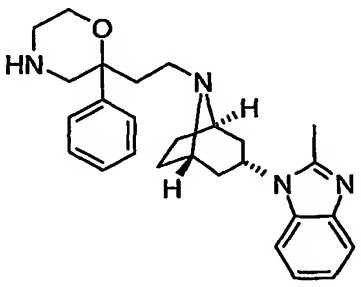
To a 0 °C solution of *tert*-butyl 2-(2-hydroxyethyl)-2-phenylmorpholine-4-carboxylate (428 mg, 1.4 mmol), dimethyl sulfoxide (4.0 mL, 56.4 mmol) and diisopropylethylamine (1.3 mL, 7.2 mmol) in 8 mL CH_2Cl_2 was added SO_3.pyr (1.4 g, 8.8 mmol) and the reaction mixture was stirred for 2h. After the addition of saturated aqueous $NaHCO_3$, the solution was extracted with $CHCl_3$, and passed through a plug of silica gel flushing with 2:1 hex:EtOAc to provide *tert*-butyl 2-(2-oxoethyl)-2-phenylmorpholine-4-carboxylate (226 mg, 53%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.60 (br. s, 1H), 7.54-7.44 (br., 2H), 7.42-7.37 (m, 2H), 7.30 (m, 1H), 4.25-4.01 (br., 1H), 3.80-3.23 (m, 5H), 3.00-2.81 (m, 2H), 2.73 (br., 1H), 1.47 (br., 9H); ESI-MS 328 (M+H).

Preparation of *tert*-butyl 2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholine-4-carboxylate



To a solution of *tert*-butyl 2-(2-oxoethyl)-2-phenylmorpholine-4-carboxylate (226.1 mg, 0.74 mmol), 1-[(1*R*,5*S*)-8-azabicyclo[3.2.1]oct-3-yl]-2-methyl-1*H*-benzimidazole dihydrochloride (272.7 mg, 0.74 mmol) and diisopropylethylamine (0.27 mL, 1.48 mmol) in 3 mL CH_2Cl_2 was added sodium triacetoxyborohydride (235.4 mg, 1.11 mmol) and the reaction mixture was stirred for 2h at room temperature. After evaporation, the residue was chromatographed (5% (2M NH_3 / MeOH) in $CHCl_3$) to provide *tert*-butyl 2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholine-4-carboxylate (329.9 mg, 84%) as an off-white foam: ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.65 (m, 1H), 7.49-7.40 (br., 2H), 7.39-7.34 (m, 2H), 7.31 (m, 1H), 7.27 (m, 1H), 7.19-7.11 (m, 2H), 4.75-4.16 (m, 2H), 3.76-3.47 (m, 3H), 3.40-3.13 (m, 4H), 2.58 (s, 3H), 2.46-2.32 (m, 2H), 2.17-1.82 (m 8H), 1.63 (m, 2H), 1.53-1.40 (br., 9H); ESI-MS 531 (M+H), 553 (M+Na).

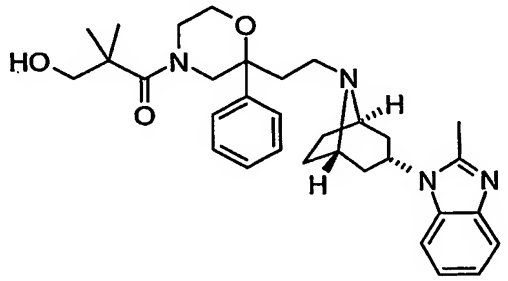
Preparation of 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole



To a solution of *tert*-butyl 2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholine-4-carboxylate (330 mg, 0.62 mmol) in 5 mL $CHCl_3$ was added 0.8 mL of a solution of 4N HCl in dioxane. After stirring at room temperature for 2 h, another 3mL 4N HCl in dioxane was added and the mixture was heated at 40 °C for 90 min. The solution was then evaporated and dried in vacuo to provide 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole trihydrochloride, which was used without further purification. ESI-MS 431 (M+H).

Example 21

Preparation of 2,2-dimethyl-3-(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)-3-oxopropan-1-ol

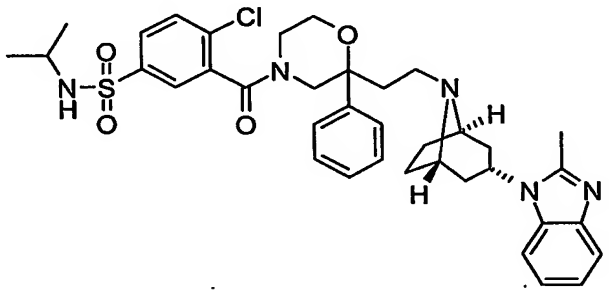


To a stirred solution of 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole trihyrdochloride (59.3 mg, 0.10 mmol), 3-hydroxy-2,2-dimethylpropanoic acid (18 mg, 0.15 mmol), and diisopropylethylamine (70μL, 0.40 mmol) in 1 mL DMF was added HATU (57 mg, 0.15 mmol). The resulting mixture was stirred at ambient temperature for 24 h and then diluted with methylene chloride and washed with saturated aqueous $NaHCO_3$. The organic phase was dried (Na_2SO_4), concentrated by evaporation, and purified by chromatography (5% (2M NH_3 / MeOH) in $CHCl_3$) to afford 2,2-dimethyl-3-(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)-3-oxopropan-1-ol (29.3 mg. 55%) as amorphous solid. ^1H NMR (400 MHz, $CDCl_3$), δ 7.65 (m, 1H), 7.44-7.26 (m, 6H), 7.19-7.10 (m, 2H), 4.80

(br., 1H), 4.69 (m, 1H), 3.75-3.37 (m, 8H), 3.35-3.16 (m, 3H), 2.58 (s, 3H), 2.46-2.34 (m, 2H), 2.13-1.84 (m, 8H), 1.68-1.59 (m, 2H), 1.23 (s, 3H), 1.02 (s, 3H); ESI-MS 531 (M+H), 553 (M+Na).

Example 22

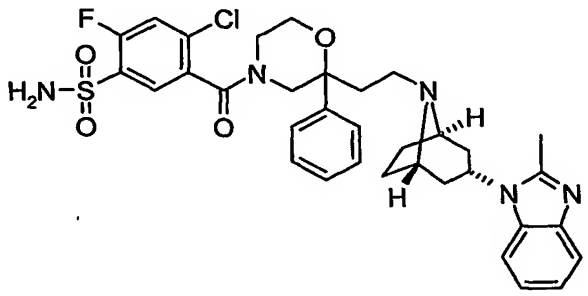
Preparation of 4-chloro-*N*-isopropyl-3-[(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)carbonyl]benzenesulfonamide



4-chloro-*N*-isopropyl-3-[(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)carbonyl]benzenesulfonamide (33.2 mg, 48%) was obtained from 2-chloro-5-[(isopropylamino)sulfonyl]benzoic acid (38 mg, 0.15 mmol), 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole trihyrdochloride (59.3 mg, 0.10 mmol) and HATU (57 mg, 0.15 mmol) following the procedure outlined in example 20. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.82-7.72 (m, 1H), 7.64 (m, 1H), 7.57-7.47 (m, 3H), 7.46-7.26 (m, 5H), 7.20-7.09 (m, 2H), 5.26-5.15 (m, 1H, rotamers), 5.06 (m, 0.5H, rotamer), 4.86 (m, 0.5H, rotamer), 4.72 (m, 1H), 3.72-3.20 (m, 7H), 3.05 (m, 1H), 2.60 (s, 3H), 2.49-1.57 (m, 11H), 1.12-1.01 (m, 6H, rotamers); ESI-MS 690 (M+H).

Example 23

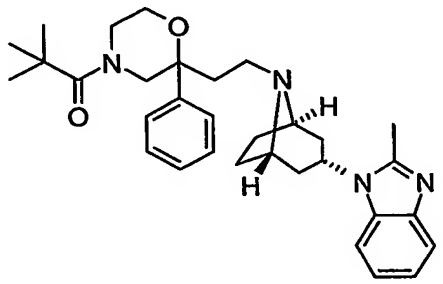
Preparation of 4-chloro-2-fluoro-5-[(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)carbonyl]benzenesulfonamide



4-chloro-2-fluoro-5-[(2-{2-[(1*R*,5*S*)-3-(2-methyl-1*H*-benzimidazol-1-yl)-8-azabicyclo[3.2.1]oct-8-yl]ethyl}-2-phenylmorpholin-4-yl)carbonyl]benzenesulfonamide (16.6 mg, 25%) was obtained from 5-(aminosulfonyl)-2-chloro-4-fluorobenzoic acid (38 mg, 0.15 mmol), 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole trihyrdochloride (59.3 mg, 0.10 mmol) and HATU (57 mg, 0.15 mmol) following the procedure outlined in example 20. ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.83 (m, 0.3H, rotamer), 7.66-7.49 (m, 2.7H, rotamers), 7.45-7.26 (m, 5H), 7.20-7.10 (m, 3H), 5.17 (m, 0.5H, rotamer), 4.92 (m, 0.5H, rotamer), 4.72 (m, 1H), 5.17 (m, 0.5H, rotamer), 4.92 (m, 0.5H, rotamer), 4.72 (m, 1H), 3.73-3.46 (m, 2H), 3.42-3.19 (m, 4H), 3.06 (m, 1H), 2.60 (m, 3H, rotamers), 249-2.37 (m, 2H), 2.17-1.79 (m, 7H), 1.71-1.61 (m, 2H); ESI-MS 666 (M+H).

Example 24

Preparation of 1-((1*R*,5*S*)-8-{2-[4-(2,2-dimethylpropanoyl)-2-phenylmorpholin-2-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole



To a solution of 2-methyl-1-{(1*R*,5*S*)-8-[2-(2-phenylmorpholin-2-yl)ethyl]-8-azabicyclo[3.2.1]oct-3-yl}-1*H*-benzimidazole trihyrdochloride (65 mg, 0.11 mmol) and Et_3N (0.08 mL, 0.55 mmol in 1 mL CH_2Cl_2 was added trimethylacetyl chloride (0.02 mL, 0.17 mmol), and the reaction was stirred for 24 h. After washing with saturated aqueous $NaHCO_3$, the organic phase was isolated, dried (Na_2SO_4), concentrated and chromatographed (5% (2M NH_3 / MeOH) in $CHCl_3$) to afford 1-((1*R*,5*S*)-8-{2-[4-(2,2-dimethylpropanoyl)-2-phenylmorpholin-2-yl]ethyl}-8-azabicyclo[3.2.1]oct-3-yl)-2-methyl-1*H*-benzimidazole (61 mg, quant.) as a film: ^1H NMR (400 MHz, $CDCl_3$), δ 7.68 (m, 1H), 7.45-7.40 (m, 2H), 7.38-7.27 (m, 3H), 7.19-7.11 (m, 2H), 4.77-4.63 (m, 2H), 3.76-3.54 (m, 4H), 3.40-3.20 (m, 3H), 2.59 (s, 3H), 2.48-2.36 (m, 2H), 2.15-1.85 (m, 8H), 1.69-1.59 (m, 2H), 1.19 (s, 9H); ESI-MS 515 (M+H).

BIOLOGICAL DATA

The following definitions apply:

IC_{50}	Concentration of compound that displaces 50% of radioligand
PIC_{50}	The determined IC_{50} value expressed as -log10(IC_{50})

CC-Chemokine Receptor-5 Binding by Scintillation Proximity Assay (CCR5 SPA)

Scintillation Proximity Assay for the Human CC-Chemokine Receptor, CCR-5

This protocol describes a high-throughput screen using SPA binding to identify compounds that inhibit binding of ^{125}I-MIP1α to the human CCR5 chemokine receptor.

CCR5 is a G protein-coupled receptor that binds the natural chemokine ligands, MIP1α, MIP1β and RANTES. CCR5 acts as a co-receptor with CD4 for entry of HIV-1 into human T-cells and monocytes. Chemokines also play a role in acute and chronic inflammatory processes. Chemokines are soluble proteins produced and released by a wide variety of cell types during the initial phase of a host response to a forgein substance entering the body.

Human CCR5 receptors were expressed in Chinese Hamster Ovary (CHO) cells, registration # 12025. Cells were grown in suspension and a 50 to 80 ml CCR5 cell pellet was prepared. Membrane preparation: 1) Weigh pellet; 2)Prepare an ice-cold 50mM HEPES buffer, containing 0.0025mg/ml Pefabloc, 0.0001mg/ml Pepstatin A, 0.0001mg/ml Leupeptin, 0.0001mg/ml Aprotinin (protease inhibitor cocktail), pH 7.4; 3) Homogenize pellet in 5 volumes of HEPES buffer; 4) Homogenize again with a glass homogenizer 10 to 20 strokes; 5) Centrifuge homogenate at 18,000 rpm in a F28/36 rotor using a Sorvall RC26 PlUS refrigerated Centrifuge for 30 minutes; 6) Discard supernatant and resuspend pellet in 3 volumes of HEPES buffer; 7) Homogenize and centrifuge again using steps 4-6, 2 more times; 8) Reweigh pellet and homogenize in 3X weight-to-volume of HEPES buffer; 9) Aliquot 0.5 to 1.5ml of the membrane preparation into small vials and store at –80 degrees Centigrade; 10) Determine the protein concentration of the membrane preparation using the Bio-Rad or BCA method; 11) The membrane homogenate will need to be characterized for the assay conditions a.) Protein concentration; b.) Optimal protein-to-bead ratio in SPA; and c.) Saturation curve to determine Kd and Bmax in SPA

The saturation curve binding experiment is performed by adding varying amounts of [^{125}I]MIP1α (0-8.5nM to membranes and beads in concentrations chosen from the

optimal protein/bead ratio. The data is analyzed using a non-linear curve-fitting program. The K_d and Bmax are derived from the curve.

Bacitracin 50mg/ml is dissolved in deionized water, brought to a boil for 5 minutes (to destroy protease activity) and cooled. Prepared 1ml aliquots and store at –80°C.

Protease inhibitor cocktail is prepared by dissolving 25 mg/ml of Pefabloc, 1 mg/ml of Leupeptin, 1 mg/ml of Aprotinin and 1mg/ml of Pepstatin A in 100% DMSO. The cocktail can be aliquoted and stored frozen at –20°C until needed.

Sigmacote: Any reagent bottles and reservoirs that come in contact with the radioligand are treated with Sigmacote to reduce sticking. Rinse containers with undiluted Sigmacote; rinse with deionized water several times, and allow to air dry before using.

Color Quench Assay- [^{125}I] SPA PVT color quench kit, Cat. No. RPAQ 4030, Amersham Ltd. A color quench curve is generated for each Packard TopCount and is stored in each counting protocol specific for the assay. This is done to prevent colored compounds from quenching the scintillation counts.

Compounds Preparation:
Compounds for a single concentration determination (One Shots) are delivered in 96 well Packard Optiplates containing 1 µl of compound in 100% DMSO in columns A1-H10 (80 compounds/plate). Column A11 to H11 is used for total binding (Bo) (vehicle-5 µl of the appropriate DMSO concentration) and column A12 to D12 is used for determination of nonspecific binding. No further preparation is required.

Compounds for concentration-response curves (10 points) are delivered in 96-Packard Optiplates containing 1 µl of compound in 100% DMSO in columns A1-H10. A 10-point concentration-response curve is desired for each compound with a starting high concentration of 30 µM (in the assauy final). Column A11 to H11 is used for total binding (Bo) (vehicle-5 µl of the appropriate DMSO concentration) and column A12 to D12 is used for determination of nonspecific binding. No further preparation is required

Materials:

1 M HEPES, pH 7.4, Gibco, Cat. No. 15360-080

Bacitracin, Sigma Catalog. Number. B-0125

Bovine Serum Albumin, Sigma, Cat. No. A-7888

$MgCl_2$, J.T. Baker 2444-01

$CaCl_2$, Sigma, Cat. No. C5080

MIP1α, Peprotech, Cat. No. 300-08

Sigmacote, Sigma, Cat. No. SL2

Scintillation Proximity Beads, Wheat Germ Agglutinin, Amersham, Cat No. RPNQ 0001

[^{125}I]MIP1α, NEN (#NEX298)

Packard 96 well flat-bottom Optiplate, Cat. No. 6005190

Falcon 96 well round-bottom plate, Cat. No. 3077

TOPSEAL-S, Packard, Cat. No. 6005161

Dimethyl Sulfoxide, EM Science, Cat. No. MX1458-6

Siliconized Pipette tips, Accutip, volume 200-1300uL, Cat. No. P5048-85

Siliconized Pipette tips, Bio Plas, Inc., volume 1-200uL, Cat. No. 60828-908

Reagent Reservoir, Elkay, Cat. No. 175-RBAS-000

Assay Buffer Preparation:

50 mM HEPES buffer pH 7.4, 1mM $CaCl_2$, 5mM MgCl2(this can be made ahead as a 100X stock), 1% BSA, 0.5 mg/ml Bacitracin, Protease inhibitor Cocktail (see preparation above) 100 uL/100 ml, DMSO is added to equal a final concentration of 2% per well (includes compound %DMSO) if needed.

Experimental Details:

[^{125}I]MIP1α preparation:

Prepared radioligand dilutions in container treated with Sigmacote

Reconstitute each 50 μCi vial with 0.5 ml of deionized water and store at 4°C.

Specific Activity = 2,000 Ci/mmol

Add ~60,000 cpm (0.17 nM) to each assay well in 50uL

Bo:

Make a 20% DMSO solution and add 5uls of this to each well in col A11-H11. This gives a final 2% DMSO concentration for the well when added to the 1% in the assay buffer.

NSB:

Make a stock dilution of MIP1α at 100uM using deionized water; aliquot and freeze. Dilute the MIP-1α stock solution to a concentration of 2 μM in the same 20% DMSO solution used above and add 5 μl to the wells in column A12 to D12 to give a final assay concentration of 100nM. Prepare this in a Sigmacote-treated container

Membrane and SPA Bead preparation-

The final assay concentration for the membrane is 15 μg per well. SPA beads are prepared by adding 5 ml of assay buffer to a 500 mg vial. The final concentration of SPA beads in the assay is 0.25 mg/well. Membranes and beads are premixed as a 1:1 (membrane:bead) mixture and maintained at mixture at 4°C with constant stirring. 50 μl of the mixture is added to each assay well. After all reagents have been added to the plates (total assay volume 100 μl), shake plates for 4 hours at room temperature. After 4 hours, place the plates on the TopCount in a count the plates on the TopCount for 30 sec per well using an appropriate program (i.e., one with a quench curve established for the conditions of the assay.

Data Reduction:

Data reduction is performed using the Microsoft Excel Addins Robofit or Robosage.

For single concentration assays (One Shots), the result of each test well is expressed as % inhibition using the following formula: 100*(1-(U1-C2)/(C1-C2)). Where U1 is the unknown sample in cpm observed in a particular well, C1 is the average of column 12 cpm observed in the absence of any added inhibitor, and C2 is the average of column 11 cpm observed in the presence of 1μM of MIP1α.

For concentration-response assays, the result of each test well is expressed as %B/Bo (% total specific binding) using the following formula: 100* (U1-C2)/C1-C2).

Curves were generated by plotting the %B/Bo versus the concentration and the IC_{50} is derived using the equation y=Vmax*(1-(x^n/(k^n+x^n))).

Controls and Standards:

Each plate contains 12 wells of total binding (column A11-H11). The cpm/well are averaged and are used in data reduction as value C1.Each plate also contains 4 wells of non-specific binding (wells A12-D12). The counts of these wells are averaged and used in data reduction as value C2.

A standards plate is included in each experiment. This plate contains a 14-point concentration-response curve (in triplicate) for the standard compound MIP1α at a starting concentration of 1 μM. The average historical pK_i obtained with MIP1α is 7.6.

The relevant biological response field for a single concentration (One Shots) is % inhibition. Inhibition values of >40 or >50% were considered positive responses.

The relevant biological response field for a concentration-response experiment is pK_i

HOS Assay (Also referred to as HOS-LTR-Luciferase Assay)

Materials

DMEM (GibcoBRL # 10564-011)

Trpsin-EDTA (GibcoBRL #25300-054)

Heat inactivated Fetal Bovine Serum (FBS) (Hyclone # SH30070.03)

96-well, black-walled, clear-bottom, tissue culture-treated plates (Costar # 3904)

96-well, clear-walled, clear-bottom tissue culture-treated plates (Costar # 3598)

Phosphate Buffered Saline (PBS) (GibcoBRL #14190-144)

Dimethyl Sulfoxide (DMSO) (Sigma # D2650)

Luclite Luciferase Reporter assay (Packard #6016911)

HOS-CD4.CCR5-LTR-Luciferase (Bioresource Registration # 21164): Human Osteosarcoma cell line engineered to overexpress human CD4 and human CCR5 (AIDS Repository cat# 3318) stabily transfected with HIV-1-LTR-Luciferase reporter.

Advanced Preparation

Growth and Maintenance of the HOS-CD4.CCR5-LTR-Luciferase cell line:

The cells were propagated in DMEM containing 2% FBS. Cells were split by standard trypsinization when confluency reached 80% (roughly every 2 to 3 days).

Titering of virus stocks:

HIV-1 virus stocks were titered in the assay system in order to obtain an estimate of the number of infectious particles per unit volume (described as RLU/ml). Virus stocks were diluted into DMEM containing 2% FBS and assayed as described in the "procedure" section below.

Procedure

Black-walled 96-well tissue culture plates were seeded with HOS-CD4.CCR5-LTR-Luciferase @ 0.6 to 1.2 x 10^3 cells per well in 50ul DMEM containing 2% FBS and placed in a humidified incubator @ 37°C, 5% CO_2 overnight. The following day, test compounds were titrated 4-fold at 2X the final concentration in DMEM + 2% FBS + 0.2% DMSO. 50µl of titrated compound was transferred to the HOS cells and the plates were placed in a humidified incubator at 37°C, 5% CO_2 for 1hr. An additional 60ul of 2X titrated compound was transferred to a clear-walled 96-well tissue culture plate and 60ul of HIV (diluted to appropriate m.o.i.) was added to each well and thoroughly mixed. 100ul of the HIV/compound mixture was transferred to the black-walled plates containing 100ul of cells/compound. The plates were placed in a humidified incubator at 37°C, 5% CO_2 for 72hr. Following the 72 hour incubation, 150ul of supernatant was removed and 50ul of reconstituted LUCLITE (kit reagent) was added to each well. Each plate was sealed and read in a Topcount (Packard) luminometer at 1s/well.

Data Reduction

Relative Light Units (RLU) were expressed as % control

(RLU at drug [ ] / RLU no drug)*100 = % Control

IC_{50} values were determined by any one of the following four nonlinear regression models:

y=Vmax*(1-(x^n/(K^n+x^n)))+Y2

y=Vmax*(1-(x^n/(K^n+x^n)))

y=Vmax*(1-(x/(K+x)))+Y2

y=Vmax*(1-(x/(K+x)))

Where: K is IC_{50}, Y2 is baseline, and N is Hill Coefficient

Each of the compounds of the present invention is believed to provide a pIC_{50} value of at least 5 when tested in each of the above-described assays.

Test compounds are employed in free or salt form.

All research complied with the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) and GlaxoSmithKline policy on animal use.

Although specific embodiments of the present invention have been illustrated and described in detail, the invention is not limited thereto. The above detailed description of preferred embodiments is provided for example only and should not be construed as constituting any limitation of the invention. Modifications will be obvious to those skilled in the art, and all modifications that do not depart from the spirit of the invention are intended to be included within the scope of the appended claims.